Reasons for Decline and Threats to Survival: Threats to the survival of the southern sea otter include reduced population size, increased tanker traffic, oil spills, drowning in commercial fishing nets, municipal pollution, and increased harassment caused by increased use of near-shore areas. Some evidence suggests that the decline in population growth rate is due to infectious disease.

Elevated levels of heavy metals, chlorinated hydrocarbons, PCB's, and petroleum hydrocarbons were found in sea otters in the past. Chemical contamination may also reduce suitable foraging areas (USDI-FWS 1981).

Elevated levels of mercury are known to occur in Elkhorn Slough, a tributary to Monterey Bay. Elkhorn Slough is impacted by upstream discharges of mercury. Livers collected from sea otters found dead at this location had a maximum mercury concentration of (60mg/kg) (Mark Stephenson pers comm 1998). Wren, 1986 suggested normal mercury concentrations in river otter livers were 4 mg/kg (ppm). O'Conner and Nielsen (1981) found that length of exposure was a better predictor of tissue residue level than dose in otters but higher doses produced an earlier onset of clinical signs. Acute mercury poisoning in mammals is primarily manifested in Central Nervous System damage, sensory and motor deficits, and behavioral impairment. Animals initially become anorexic and lethargic. A dose of 0.09 mg/kg body weight (2 ppm in diet) for 181 days was enough to produce anorexia and ataxia in two of three otters (Lutra candensis. Associated liver residues were 32.6 mg/kg (O'Conner and Nielsen 1981). Muscle ataxia, motor control deficits, and visual impairment develop as toxicity progresses with convulsions preceding death. River otters fed 8 ppm died within a mean time of 54 days. Associated liver concentrations were 32.3 mg/kg (ppm) (O'Conner and Nielsen 1981). Smaller carnivores are more sensitive to methylmercury toxicity than larger species as reflected in shorter times of onset of toxic signs and time to death.

DIRECT AND INDIRECT EFFECTS OF THE PROPOSED ACTION

For the purposes of this opinion the Services have conducted their effects analysis based on the potential for the numeric criteria to result in effects to the aquatic ecosystem and the species that are dependent on its function for their survival and recovery. While 126 priority pollutants are addressed within the CTR, the Services have focused upon the numeric criteria for selenium, mercury, pentachlorophenol, cadmium and formula based criteria for metals on a dissolved basis as the most problematic for listed species and critical habitat. The Services have prepared this analysis of criteria for priority pollutants based on: (1) the adequacy of the proposed aquatic life criteria, including the necessity of wildlife criteria where aquatic life criteria are not sufficiently protective of wildlife; (2) the toxic effects to listed species or surrogates which may occur at proposed criteria concentrations; (3) the bioaccumulative nature of the priority pollutants at issue; and (4) the potential for interactive effects of pollutants at the proposed criteria concentrations. In some cases, such as mercury, if the aquatic life criteria were not protective and the human health criteria were lower, the adequacy of the human health numeric criteria to protect aquatic life was also considered.

Our analysis of criteria assessed whether there was the potential for toxicity that would affect listed species to occur at concentrations at or below the proposed criteria concentrations in water. EPA has stipulated that the promulgation of the CTR is solely for the purpose of providing the State of California with criteria. Although the Services recognize that criteria are sometimes not met within some California waterbodies and that implementation and enforcement issues also determine the degree of protection, the analysis within this opinion assesses the degree of protection likely to be afforded to listed species by the CTR if concentrations of toxic pollutants allowable by the proposed CTR are achieved. While EPA has not specifically proposed any wildlife criteria as part of the CTR, the Services are required to evaluate the degree of protection afforded to listed wildlife species by the proposed criteria for all California waterbodies.

The Services have evaluated the effects of the proposed action based on the assumptions that: (1) the proposed numeric criteria will apply throughout the geographic distribution of the species; and (2) the ambient concentrations of constituents could rise to the concentrations allowed by the numeric criteria proposed by EPA. Included in these findings are the Services' analysis of the demonstrated potential for adverse effects to occur to species at or below the proposed criteria concentrations, the likelihood of these problematic concentrations being achieved within the range of the species, and the degree to which these adverse effects will impact the species' environmental baseline.

The Services in the development of this final biological opinion have used the same rationale for evaluating effect thresholds of criteria as previously presented in our April 10, 1998, and April 9, 1999, draft biological opinions. That rationale is presented in the "Consultation History" section of this document. The Services based the following effects section on EPA's August 5, 1997, proposed CTR. Since that time EPA has modified the proposed action as presented in EPA's December 16, 1999, letter to the Services, and memorialized in the "Description of the Proposed Action" section of this document. The subsequent conclusions contained in this document are contingent on EPA's implementation of these modifications.

Selenium

Assessment of Adequacy of Proposed Selenium Criteria to protect listed species

Chronic Aquatic Life Criterion for Selenium

The Services find that the chronic aquatic life criterion for selenium proposed in the CTR does not protect listed fish and wildlife dependent on the aquatic ecosystem for development and/or foraging. The Federal Register notice for the proposed rule (EPA 1997c) states that the chronic criterion of 5 µg/L for selenium (derived in 1987) continues to be scientifically valid and protective of aquatic life. However, nearly every major review of experimental and field data conducted over the past decade has concluded that a chronic criterion of 5 µg/L is not fully protective of fish and wildlife resources. The list of scientific reviews known to the Service that contradict the 5 µg/L chronic criterion includes: Lemly and Smith (1987), Davis *et al.* 1988,

Lillebo *et al.* (1988), UC Committee (1988), DuBowy (1989), Johns 1989, Lemly 1989, U.S. Dept. of Interior and California Resources Agency (1990), Sorensen (1991), Environment Canada (1991), Pease *et al.* (1992), Peterson and Nebeker (1992), CH2M HILL *et al.* (1993), Emans et al. 1993, Lemly (1993a), Lemly (1993b), CAST (1994), Gober (1994), Maier and Knight (1994), New Mexico (1994), California Regional Water Board (1995), Lemly (1995), Seiler and Skorupa (1995), California Regional Water Board (1996), Lemly (1996a), Lemly (1996b), Ohlendorf (1996), Roux et al. (1996), Skorupa *et al.* (1996), Van Derveer and Canton (1997), Engberg *et al.* (1998), Skorupa (1998), Naftz and Jarman (1998), Stephens and Waddell (1998), Adams *et al.* (1998), Seiler and Skorupa (In Press), and Hamilton and Lemly, 1999. Each of these reviews, incorporates the findings from numerous individual studies, for example, Skorupa *et al.* (1996) cite results from about 200 individual studies. In aggregate, the weight of scientific evidence supporting a chronic criterion for selenium of $\leq$ 2 µg/L is now overwhelming.

As early as 1991, the evidence available in the scientific literature was sufficient for Canada to issue a national water quality guideline stipulating that the concentration of total selenium should not exceed 1 µg/L (Environment Canada 1991). Based on data collected by the U.S. Department of Interior's National Irrigation Water Quality Program (NIWQP) from 26 study areas in 14 western states (including 5 California study areas), a 5 µg/L chronic criterion for selenium is only 50-70 percent protective (Adams *et al.* 1998; Seiler and Skorupa, In Press), as opposed to the 95 percent level of protection that EPA's national water quality criteria are intended to achieve (Stephan *et al.* 1984). The Service believes the NIWQP data suggest that on a dissolved basis a criterion of 1 µg/L would be required to achieve 95 percent protection, which is approximately equivalent to a 2 µg/L criterion on a total recoverable basis (Peterson and Nebeker 1992).

Acute Aquatic Life Criterion for Selenium

The Services find that the speciation-weighted acute criterion for selenium proposed in the CTR does not protect listed fish and wildlife dependent on the aquatic ecosystem for development and/or foraging. The EPA proposed changing the acute criterion for selenium from 20 µg/L (total recoverable) to a speciation-weighted criterion based on the relative concentrations of selenite, selenate, and all other forms of selenium found in a particular water body. Depending on the specific water body in question, this proposed acute criterion for selenium could range from 12.8 µg/L (if 100 percent selenate were present) to 185.9 µg/L (if 100 percent selenite were present). A 20 µg/L (total recoverable) acute site-specific criterion was promulgated in the NTR (and would not be changed by the CTR) and applies to the following water bodies in California: San Francisco Bay upstream to and including Suisun Bay, Sacramento-San Joaquin Delta, Mud Slough, Salt Slough, San Joaquin River, and Sack Dam to the mouth of the Merced River. The Services believe that the promulgation of the proposed speciation weighted acute criterion for selenium in the CTR would not afford adequate protection to listed species because: (1) selenium bioaccumulates rapidly in aquatic organisms and a single pulse of selenium ($\geq$10 µg/L) into aquatic ecosystems could have lasting ramifications, including elevated selenium concentrations in aquatic food webs (Maier *et al.* (in press); Hansen's Biological Consulting et al. 1997a, 1997b, 1998; Hanson et al. 1996; Tulare Lake Drainage District 1996); (2) EPA's speciation-

weighted criterion assumes that selenate is more toxic than selenite, which is the reverse of what has been found in most acute selenium toxicity studies; (3) and the site-specific criterion of 20 µg/L promulgated in the NTR may fail to adequately protect aquatic-dependent fish and wildlife (Lemly 1997; Maier *et al*. 1998; Hansen's Biological Consulting et al. 1997a, 1997b, 1998; Hanson et al. 1996; Tulare Lake Drainage District 1996). For example, in February 1995, the Tulare Lake Drainage District established a flow-thru compensation wetland. Although the water supplied to the wetland was generally managed to keep its selenium content at or below about 2-3 µg/L, a pulse of 23 µg/L was documented on March 29, 1995 (Tulare Lake Drainage District 1996; Hanson et al. 1996). Three months later (June 20, 1995), and without any additional selenium pulses, avian eggs sampled at the site contained up to 6.2 µg/g Se which exceeds the embryotoxic risk threshold reported in Skorupa (1998). In June 1995, 12% of sampled eggs exceeded 6 µg/g Se which very plausibly may have been linked to the late March pulse of 23 µg/L Se that passed through the system. Additional support for a "pulse-effect" hypothesis, is provided by monitoring data for 1996-1998. In each of those three years, water supplied to the wetland was never documented to exceed 2.8 to 4.2 µg/L Se, and in all three years, in the absence of a $\geq$ 10 µg/L Se pulse, none of the avian eggs collected at the site exceeded the embryotoxicity threshold of 6 µg/g Se (Hansen's Biological Consulting et al. 1997a, 1997b, 1998).

The Services believe the acute toxicity data that were reviewed and compiled in Maier *et al*. (1987), Lillebo *et al*. (1988), Moore *et al*. (1990), and Skorupa *et al*. (1996) should be incorporated by EPA into the database that is employed for deriving a speciation-weighted acute criterion. These sources, and field studies (cf. Skorupa 1998), unanimously indicate that a lower criterion is warranted for selenite-dominated waters than for selenate-dominated waters (the reverse of the currently proposed weighting formula). Canton (1996) suggested that EPA's erroneous acute toxicity weighting of selenate versus selenite is the result of the influence of unusual outlier data for one taxon, *Gammarus*, and the small data base for acute toxicity testing of selenate. This suggests that only strictly matched comparative data should be used to derive a speciation-weighted acute criterion for selenium.

Hazards of Selenium

Selenium Sources

Selenium, a semi-metallic trace element with biochemical properties very similar to sulfur, is widely distributed in the earth's crust, usually at trace concentrations (<1 µg/g, ppm; e.g., Wilber 1980; Eisler 1985). Some geologic formations, however, are particularly seleniferous (e.g., Presser and Ohlendorf 1987; Presser 1994; Presser et al. 1994; Piper and Medrano 1994; Seiler 1997; Presser and Piper 1998) and when disturbed by anthropogenic activity provide pathways for accelerated mobilization of selenium into aquatic ecosystems. Abnormally high mass-loading of selenium into aquatic environments is most typically associated with the use of fossil fuels, with intensive irrigation and over-grazing of arid lands, and with mining of sulfide ores (Skorupa 1998). Intensive confined livestock production facilities and municipal wastewater treatment

plants may also contribute to accelerated mass-loading of selenium into surface water bodies.

The use of fossil fuels can result in accelerated mass-loading of selenium into aquatic environments via the leaching of coal-mining spoils and/or overburden, via disposal of process wastewater from oil refineries, via downwind drift and deposition from industrial-scale coal combustion, and via aquatic disposal and/or leaching of fly ash from coal-fired electric power plants (Lemly 1985; Skorupa 1998). Agricultural irrigation over large areas of the western United States causes accelerated leaching of selenium from soils into groundwater. Natural and anthropogenic discharge of subsurface agricultural drainage water to surface waters is a major pathway for the mass-loading of selenium into aquatic ecosystems (Presser et al. 1994; Presser 1994; Seiler 1997; Presser and Piper 1998; Skorupa 1998). Overgrazing of high-gradient watersheds can cause accelerated erosion of seleniferous soils and detrital litter into surface waters, but no case studies of this pathway have been systematically documented. Mining of sulfide ores (other than coal) such as uranium, copper, bentonite, and phosphoria is also a common source of artificially mobilized selenium. Selenium concentrations as high as 4,500 µg/g (ppm) have been reported in the overburden from uranium mining (USDI-BOR/FWS/GS/BIA 1998). Leachates from phosphoria overburden drains have been documented to contain > 2,000 µg/L (ppb) selenium and to have caused selenium toxicosis among livestock in downstream pastures where creeks contained 300 µg/L waterborne selenium (Talcott and Moller 1997).

The recent rapid expansion of high-density confined livestock production facilities pose yet another potential pathway for accelerated mobilization of selenium into aquatic ecosystems. Most commercial livestock feeding operations (and dairies) add supplemental selenium to the feeds and Oldfield (1994) reported that liquid manure pits beneath feed barns contained 50-150 µg/L of selenium. Unlike human wastes, animal wastes are often discharged to surface water bodies without any prior waste treatment. The biochemistry of selenium in liquid manure might be unique compared to other artificial mobilization pathways (CAST 1994), but this has not been confirmed. The environmental fate of "feed barn" selenium has not been systematically researched to date. Solid manure is also a common ingredient in commercial fertilizers and can reach surface waters via drift during fertilizer application , equipment cleansing, and downslope drainage of leachates. Although most municipal wastewater treatment systems process nonseleniferous wastewater (Westcot and Gonzalez 1988), on a regional and local basis mass-loading of selenium to surface waters from public wastewater treatment facilities can be ecologically significant (Pease *et al*. 1992; CRWQCB 1995). This may be of particular concern where constructed wetlands, that attract use by wildlife, are a component of the water treatment process.

Toxicity

For vertebrates, selenium is an essential nutrient (Wilber 1980). Inadequate dietary uptake (food and water) of selenium results in selenium deficiency syndromes such as reproductive impairment, poor body condition, and immune system dysfunction (Oldfield 1990; CAST 1994).

However, excessive dietary uptake of selenium results in toxicity syndromes that are similar to the deficiency syndromes (Koller and Exon 1986). Thus, selenium is a "hormetic" chemical, i.e., a chemical for which levels of safe dietary uptake are bounded on both sides by adverse-effects thresholds. Most essential nutrients are hormetic; what distinguishes selenium from other nutrients is the very narrow range between the deficiency threshold and the toxicity threshold (Wilber 1980; Sorensen 1991). Nutritionally adequate dietary uptake (from feed) is generally reported as 0.1 to 0.3 µg/g (ppm) on a dry feed basis, whereas, the toxicity threshold for sensitive vertebrate animals is generally reported as 2 µg/g (ppm). That dietary toxicity threshold is only one order-of-magnitude above nutritionally adequate exposure levels (see review in Skorupa *et al*. 1996; USDI-BOR/FWS/GS/BIA 1998).

Hormetic margin-of-safety data suggest that environmental regulatory standards for selenium should generally be placed no higher than one order of magnitude above normal background levels (unless there are species-specific and site-specific data to justify a variance from the general rule). For freshwater ecosystems that are negligibly influenced by agricultural or industrial mobilization of selenium, normal background concentrations of selenium have been estimated as 0.25 µg/L (ppb; Wilber 1980), 0.1-0.3 µg/L (ppb; Lemly 1985), 0.2 µg/L (ppb; Lillebo *et al*. 1988), and 0.1-0.4 µg/L (ppb; average <0.2, Maier and Knight 1994). These estimates suggest, based on a margin-of-safety line of reasoning, that the aquatic life chronic criterion for selenium should be *no higher* than 4 µg/L (= 10-times the upper boundary for normal background), and that a criterion of 2 µg/L would be most consistent with the central tendency value (0.2 µg/L) for normal background levels of waterborne selenium and a one order-of-magnitude margin of safety.

Direct Waterborne Contact Toxicity

Selenium occurs in natural waters primarily in two oxidation states, selenate (+6) and selenite (+4). Waters associated with various fossil-fuel extraction, refining, and waste disposal pathways contain selenium predominantly in the selenite (+4) oxidation state. Waters associated with irrigated agriculture in the western United States contain selenium predominantly in the selenate (+6) oxidation state. Based on traditional bioassay measures of toxicity (24- to 96-hour contact exposure to contaminated water *without* concomitant dietary exposure), selenite is more toxic than selenate to most aquatic taxa (e.g., see review in Moore *et al*. 1990).

Most aquatic organisms, however, are relatively insensitive to waterborne contact exposure to either dissolved selenate or dissolved selenite, with adverse-effects concentrations generally above 1,000 µg/L (ppb). By contrast, waterborne contact toxicity for selenium in the form of dissolved seleno-amino-acids (such as selenomethionine and selenocysteine) has been reported at concentrations as low as 3-4 µg/L for striped bass (*Morone saxitilis)* (ppb; Moore *et al*. 1990). It would be expected, however, that at a chronic standard of 5 µg/L (ppb) *total selenium* the concentration of dissolved seleno-amino-acids would be substantively below 3-4 µg/L (ppb) because seleno-amino-acids usually make up much less than 60-80 percent of *total dissolved selenium* in natural waters. For example, it was estimated that organoselenium made up only 4.5

percent of the total dissolved selenium in highly contaminated drainage water from the San Joaquin Valley (Besser *et al*. 1989). Under most circumstances, a 5 µg/L chronic criterion should be protective of aquatic life *with regard to direct contact toxicity*. Selenium, however, is bioaccumulative and therefore direct contact exposure is only a minor exposure pathway for aquatic organisms (e.g., see review by Lemly 1996a).

Bioaccumulative Dietary Toxicity

Although typical concentrations of different chemical forms of selenium would be unlikely to cause direct contact toxicity at an aquatic life chronic standard of 5 µg/L (ppb), as little as 0.1 µg/L of dissolved selenomethionine has been found sufficient, via bioaccumulation, to cause an average concentration of 14.9 µg/g (ppm, dry weight) selenium in zooplankton (Besser *et al*. 1993), a concentration that would cause dietary toxicity to most species of fish (Lemly 1996a). Based on Besser *et al*. (1993) bioaccumulation factors (BAFs) for low concentrations of selenomethionine, as little as 6 ng/L (ppt) of dissolved selenomethionine would be sufficient to cause foodchain bioaccumulation of selenium to concentrations exceeding toxic thresholds for dietary exposure of fish and wildlife. Thus, at a chronic aquatic life standard of 5 µg/L (ppb) as *total selenium*, if more than 0.1 percent of the total dissolved selenium were in the form of selenomethionine, foodchain accumulation of selenium to levels sufficient to cause dietary toxicity in sensitive species of fish and birds would occur. For highly contaminated water (100-300 µg/L selenium) in the San Joaquin Valley about 4.5 percent of all dissolved selenium was in the form of organoselenium (Besser *et al*. 1989). Unfortunately, relative concentrations of seleno-amino-acids have not been determined in the field in California for waters where total selenium is found in the critical 1-5 µg/L range. Further research is required to characterize typical proportions of seleno-amino-acids in waters containing 1-5 µg/L (ppb) *total selenium*.

Based on waters containing 1-5 µg/L (ppb) *total selenium*, composite bioaccumulation factors (defined as: the total bioaccumulation of selenium from exposure to a composite mixture of different selenium species measured only as *total selenium*) for aquatic foodchain items (algae, zooplankton, macroinvertebrates) are typically between 1,000 and 10,000 (on dry weight basis; Lillebo *et al*. 1988; Lemly 1996a). Therefore, based on risk from bioaccumulative dietary toxicity, a generic aquatic life chronic criterion in the range of 0.2 to 2 µg/L (ppb) would be justified (where generic is defined as: the absence of site-specific and species-specific toxicological data). In fact, based on an analysis of bioaccumulative dietary risk and a literature database, Lillebo *et al*. (1988) concluded that a chronic criterion of 0.9 µg/L (ppb) for *total selenium* is required to protect fish from adverse toxic effects. Furthermore, Peterson and Nebeker (1992) applied a bioaccumulative risk analysis to semi-aquatic wildlife taxa and concluded that a chronic standard of 1 µg/L (ppb) for *total selenium* was warranted. Most recently, Skorupa (1998) has compiled a summary of field data that includes multiple examples of fish and wildlife toxicity in nature at waterborne selenium concentrations below 5 µg/L (ppb), supporting the criteria recommendations of Lillebo *et al*. (1988) and Peterson and Nebeker (1992). Furthermore, a recently concluded regional survey of irrigation related selenium mobilization in the western United States, conducted jointly by several agencies of the U.S.

Department of the Interior over a ten-year period, found that at 5 µg/L total Se in surface waters about 60% of associated sets of avian eggs exceeded the toxic threshold for selenium, i.e., that 5 µg/L Se was only about 40% protective against excessive bioaccumulation of selenium into the eggs of waterbirds (Seiler and Skorupa, In Press).

Interaction Effects Enhancing Selenium Toxicity

Toxic thresholds for fish and wildlife dietary exposure to selenium have been identified primarily by means of controlled feeding experiments with captive animals (e.g., see reviews by NRC 1980, 1984, 1989; Heinz 1996; Lemly 1996a; Skorupa *et al*. 1996; USDI-BOR/FWS/GS/BIA 1998). Such experiments are carefully designed to isolate the toxic effects of selenium as a *solitary stressor*. Consequently, the toxic thresholds identified by such studies are prone to overestimating the levels of selenium exposure that can be tolerated, without adverse effects, in an environment with *multiple stressors* as is typical of the real ecosystems (Cech et al. 1998). There are at least three well-known multiple-stressor scenarios for selenium that dictate a very conservative approach to setting water quality criteria for aquatic life:

1. Winter Stress Syndrome - More than 60 years ago it was first discovered in experiments with poultry housed in outdoor pens that dietary toxicity thresholds were lower for experiments done in the winter than at other times of the year (Tully and Franke 1935). More recently this was confirmed for mallard ducks (*Anas platyrhynchos*) by Heinz and Fitzgerald (1993). Lemly (1993b), studying fish, conducted the first experimental research taking into account the interactive effects of winter stress syndrome and confirmed that such effects are highly relevant even for waters containing <5 µg/L (ppb) selenium. Consequently, Lemly (1996b) presents a general case for winter stress syndrome as a critical component of hazard assessments. It can be further generalized that any metabolic stressor (cold weather, migration, smoltification, pathogen challenge, etc.) would interact similarly to lower the toxic thresholds for dietary exposure to selenium. Based on a comparison of results from Heinz and Fitzgerald (1993) and Albers *et al*. (1996), the dietary toxicity threshold in the presence of winter stress was only 0.5-times the threshold level for selenium as a solitary stressor. Thus, it appears that criteria based on single-stressor data should be reduced by at least a factor of two. The proposed chronic criterion for selenium of 5 µg/L (ppb) is based, in part, on field data from Belews Lake (EPA 1987a), presumably including multiple stressors as typically encountered in nature. However, as recently noted in a presentation by Dr. Dennis Lemly to the EPA Peer Consultation Committee on selenium (EPA 1998:3-5), EPA's 5 µg/L (ppb) criterion was based on the erroneous presumption that the Hwy. 158-Arm of Belews Lake was "unaffected." Dr. Lemly argues that in fact multiple lines of evidence indicate adverse effects of selenium on fish in the Hwy. 158-Arm of Belews Lake at concentrations of 0.2-4 µg/L (ppb). Dr. Lemly concludes that the true (multiple stressor) ". . . threshold for detrimental impacts [at Belews Lake] is well below 5 µg/L."

2. Immune System Dysfunction - Also more than 60 years ago, it was first noted that chickens exposed to elevated levels of dietary selenium were differentially susceptible to pathogen challenges (Tully and Franke 1935). More recently this was confirmed for mallard ducks by

Whiteley and Yuill (1989). Numerous other studies have confirmed the physiological and histopathological basis for selenium-induced immune system dysfunctions in wildlife (Fairbrother and Fowles 1990; Schamber *et al.* 1995; Albers *et al.* 1996). Based on Whiteley and Yuill's (1989) results, *in ovo* exposure of mallard ducklings to as little as 3.9 µg/g (ppm dry weight basis) selenium was sufficient to significantly increase mortality when ducklings were challenged with a pathogen. The lowest confirmed *in ovo* toxicity threshold for selenium as a solitary stressor is 10 µg/g (ppm dry weight basis; Heinz 1996, reported as 3 µg/g wet weight basis and about 70% moisture). In this case the multiple-stressor toxicity threshold is only 0.39-times the threshold level for selenium as a solitary stressor. Based, in part, on the solitary stressor toxic threshold reported by Heinz (1996) for mallard eggs, Adams et al. (1998) concluded that 6.77 µg/L Se would be 90% protective against excessive bioaccumulation of selenium into avian eggs. Therefore based on a pathogen challenge multiple-stressor scenario a protective water quality criterion would be (0.39) X (6.77 µg/L) = 2.6µg/L (ppb). Again, the multiple-stressor threshold would appear to be well below the proposed chronic criterion of 5 µg/L (ppb).

3. Chemical Synergism - Multiple stressors can also consist of other contaminants. For example, Heinz and Hoffman (1998) recently reported very strong synergistic effects between dietary organo-selenium and organo-mercury with regard to reproductive impairment of mallard ducks. The experiment of Heinz and Hoffman (1998) did not include selenium treatments near or below the threshold for diet-mediated reproductive toxicity and therefore no ratio of single-stressor versus multiple-stressor threshold levels is available. A field study involving 12 lakes in Sweden, however, found that in the presence of threshold levels of mercury contamination, the waterborne threshold for selenium toxicity was about 2.6 µg/L (ppb; see review in Skorupa 1998; and review in USDI-BOR/FWS/GS/BIA 1998). The Swedish lakes' result is in agreement with multiple-stressor derived criteria suggested above for winter stress and for pathogen challenge as multiple stressors. Based on the Swedish lakes study, which encompassed 98 different lakes, Lindqvist et al. (1991) concluded, "It is important not to dose so that Se concentrations in water rise above about 1 to 2 µg Se/L." Likewise, Meili (1996) concluded that, "The results [of the Swedish Lakes studies] suggest that a selenium concentration of only 3 µg/L can seriously damage fish populations."

At least one field study of birds also provides circumstantial evidence of lowered toxicity thresholds for selenium-induced reproductive impairment in the presence of mercury contamination (Henny and Herron 1989).

Environmental Partitioning and Waterborne Toxicity Thresholds

Risk management via water concentration-based water quality criteria is an inherently flawed process for selenium (Pease *et al.* 1992; Taylor *et al.* 1992, 1993; Canton 1997). The process is flawed because the potential for toxic hazards to fish and wildlife is determined by the rate of mass loading of selenium into an aquatic ecosystem and the corresponding environmental partitioning of mass loads between the water column, sediments, and biota (food chain). However, a water column concentration of selenium can be an imperfect and uncertain measure of mass

loading and foodchain bioaccumulation. For example, a low concentration of waterborne selenium can occur because mass loading into the system is low (= low potential for hazard to fish and wildlife) or because there has been rapid biotic uptake and/or sediment deposition from elevated mass loading (= high potential for hazard to fish and wildlife). Toxicity to fish and wildlife is ultimately determined by how much selenium is partitioned into the food chain. Therefore, water quality criteria are useful guides for risk management only to the extent that they protect aquatic food chains from excessive bioaccumulation of selenium. As evidenced by the literature cited above, a water quality chronic criterion of 2 µg/L will protect aquatic food chains from excessive bioaccumulation under most permutations of environmental and anthropogenic factors (i.e., the probability of adverse effects is sufficiently low). However, several examples of potentially hazardous foodchain bioaccumulation of selenium at waterborne selenium concentrations <2 µg/L are known from California (Maier and Knight 1991; Pease *et al*. 1992; Luoma and Linville 1997; San Francisco Estuary Institute [SFEI] 1997a; Setmire *et al*. 1990, 1993; Bennett 1997) and elsewhere (Birkner 1978; Lemly 1997; Hamilton 1998). To substantively decrease the regulatory uncertainty of water quality criteria for selenium, ultimately a criterion-setting protocol will have to be formulated that links risk management and regulatory goals directly to aquatic food chain contamination (for example, see Taylor *et al*. 1992, 1993).

Selenium Summary

A variety of conceptual bases for deriving a generally applicable chronic water quality criterion for selenium that is protective of fish and wildlife have been presented above with the following results:

Hormetic Margin of Safety Basis: 1-4 µg/L (ppb), with 2 µg/L (ppb) being most consistent with central tendency data.

Waterborne Exposure Only Basis (= Traditional Bioassay Testing): 3-4 µg/L (ppb) for selenium in the form of seleno-amino-acids (e.g., selenomethionine); current EPA chronic criterion of 5 µg/L (ppb) adequate for selenium as inorganic ions (e.g., selenite and selenate).

Bioaccumulative Dietary Exposure Basis (with Selenium as solitary stressor):
0.2-2.0 µg/L (ppb), with 0.9-1.0 µg/L (ppb) supported by the two most detailed reviews to date.

Winter Stress Syndrome Multiple Stressor Basis: ". . . well below . . . " 5 µg/L (ppb).

Pathogen Challenge Multiple Stressor Basis: 2.6 µg/L (ppb).

Mercury Synergism Multiple Stressor Basis: 2-3 µg/L (ppb).

Overwhelmingly, the available body of scientific evidence (the majority of which has been produced subsequently to EPA's 1987 criterion derivation for selenium) consistently supports a chronic criterion of 2 µg/L (ppb) for the protection of sensitive taxa of fish and wildlife. Even a

criterion of 2 µg/L, however, can fail to be protective in specific cases where water column contamination with selenium fails to accurately reflect food chain contamination. There is a strong need for developing a method to link criteria directly to food chain contamination. In the absence of site-specific and species-specific data regarding the sensitivity of particular species and/or populations, a general criterion of at least 2 µg/L is required to assure adequate protection of threatened and endangered species of fish and wildlife. This is especially warranted considering the steep response curves for selenium (Hoffman *et al*. 1996; Lemly 1998; Skorupa 1998) and the well-demonstrated potential for selenium-facilitated pathogen susceptibility that can rapidly extirpate entire populations of fish and wildlife via epizootic events.

Summary of Effects of Selenium to Listed Species

Birds

The Services conclude that selenium poisoning of birds foraging in aquatic systems may occur at or below concentrations permissible under the aquatic life criteria proposed in the CTR. The effects of selenium poisoning on avian species include: gross embryo deformities, winter stress syndrome, depressed resistance to disease due to depressed immune system function, reduced juvenile growth and survival rates, mass wasting, loss of feathers (alopecia), embryo death, and altered hepatic enzyme function. In addition the interactive effects between mercury and selenium produce super-toxic effects greater than effects of each compound individually that may include embryo deformities, embryo death, reduced juvenile survival, behavioral abnormalities, depressed immune response, mass wasting, and mortality. It is the aggregation of these effects that the Service believes are likely to adversely affect the bald eagle, California clapper rail, California brown pelican, California least tern, light-footed clapper rail, marbled murrelet, and the Yuma clapper rail, based on the potential for these species to be impacted by elevated levels of selenium through their dietary habits, dependence on the aquatic ecosystem, and their limited distribution.

A species which the Service believes will not be adversely affected is the snowy plover. The coastal populations of the snowy plover have a significant terrestrial component to their diet which likely provides dietary dilution of aquatic system selenium exposures, and have been shown on a species-specific basis to be very tolerant to selenium exposure.

Aleutian Canada Goose: As herbivorous waterbirds, with a fairly unique ecological niche, all forms of Canada geese can be expected to be extremely sensitive to dietary exposure to selenium. The basis for this sensitivity was presented via energetic modeling by DuBowy (1989) for American coots (*Fulica americana*), another herbivorous species of waterbird. Herbivorous birds consume such a large bulk of vegetation to meet caloric requirements (compared to birds feeding on high caloric dense animal matter) that their mass dosing of selenium can be very high even though the diet contains a lower concentration of selenium than normally considered toxic for other species.

A field study of Canada geese (*Branta canadensis*) in Wyoming (See *et al*. 1992) reported widespread reproductive failure among geese with relatively low exposure to selenium (eggs averaging 5-10 µg/g Se). If selenium caused the observed reproductive failure in Wyoming as the authors of the report believed, but which was not well established (Skorupa 1998), and if as little as 5 µg/g Se in eggs of geese is reproductively hazardous, then a 5 µg/L water quality criterion for selenium would fail to protect geese (most avian species exhibit water to egg bioaccumulation factors of at least 1,000-fold; Ohlendorf *et al*. 1993, Skorupa *et al*. unpubl. data).

The Aleutian Canada goose would be most likely to encounter selenium-contaminated vegetation in wetlands. In contrast to breeding geese, which would be expected to feed in the wetlands used for nesting, wintering Aleutian Canada geese in California feed primarily in upland crops and fallow fields. Thus, it is expected that exposure to wetland vegetation would be rare for the Aleutian Canada goose while wintering in California and that selenium standards for such wetlands are not an important issue for the survival and recovery of this subspecies.

Bald Eagle: At least two citations in the selenium literature provide a basis for doubting that a chronic selenium standard of 5 µg/L (ppb) would be sufficiently protective of bald eagles. Lillebo *et al*. (1988) derived levels of selenium to protect various species of waterbirds. Based on an analysis of bioaccumulation dynamics and an estimated critical dietary threshold for toxicity of 3 µg/g, they concluded that piscivorous birds would be at substantially greater risk of toxic exposure than mallards (*Anas platyrhynchos*). The calculated water criterion to protect piscivorous birds was 1.4 µg/L (ppb) as opposed to 6.5 µg/L (ppb) for mallards. The proposed CTR criterion of 5 µg/L (ppb) is more than 3-times the calculated criterion for piscivorous birds. It should also be noted that the 6.5 µg/L (ppb) calculated criterion for mallards exceeds the actual threshold point for ducks in the wild which is somewhere below 4 µg/L (ppb) (Skorupa 1998). Thus, the 1.4 µg/L (ppb) calculated criterion for piscivorous birds may be biased high compared to the wild as well.

Applying an energetics modeling approach, modified from the Wisconsin Department of Natural Resources, Peterson and Nebeker (1992) calculated a chronic criterion specifically for Bald eagles. Peterson and Nebeker's estimate of a protective criterion is 1.9 µg/L (ppb). Again, the estimate is below the CTR proposed criterion of 5 µg/L (ppb). However, Peterson and Nebeker calculated a mallard criterion (2.1 µg/L; ppb) that was much closer to their Bald eagle criterion than Lillebo *et al.'s* results would suggest. Peterson and Nebeker's mallard criterion is consistent with real-world data (cf. Skorupa 1998) and therefore their bald eagle criterion may also be reliable.

Consequently, best available evidence suggests that widespread expansion of aquatic habitats containing > 1.9 µg/L (ppb) selenium, as could occur with a criterion of 5 µg/L (ppb), could put substantial numbers of California's bald eagles at risk of toxic effects of selenium.

California Brown Pelican: As a large-bodied piscivorous bird, much of the discussion provided above for the bald eagle regarding the inadequacy of the CTR-proposed selenium criteria may

also apply to the California brown pelican. Consequently, until species-specific data are collected or species-specific modeling is conducted for the California brown pelican, a selenium criterion on the order of 1.4 µg/L (ppb) (generic piscivorous bird model; Lillebo *et al.* 1988) to 1.9 µg/L (ppb) (bald eagle model; Peterson and Nebeker 1992) must be viewed as the applicable guidance for protection of California brown pelicans from selenium poisoning. The CTR-proposed criterion of 5 µg/L (ppb) must therefore be viewed as unprotective of California brown pelicans foraging in the Salton Sea and enclosed bays and estuaries in the State of California.

In the 1990's there have been at least 4 major avian epizootic events at California's Salton Sea, including suspected algal toxin poisoning of more than 175,000 eared grebes (in two episodes), botulism poisoning of about 15,000 piscivorous birds (including more than 1,400 Brown Pelicans) and a Newcastle's disease outbreak in a cormorant colony (Bennett 1994; USGS 1996; USDI-FWS 1997c). Normal selenium nutrition is a well-documented requirement for the proper functioning of avian and fish immune systems (e.g., Larsen *et al.* 1997; Wang and Lovell 1997). Deficient and toxic levels of selenium equally cause immune system dysfunctions (e.g., Larsen *et al.* 1997) and for 60 years it has repeatedly been demonstrated clinically that birds and fish suffering from selenium-induced immune dysfunctions are hypersensitive to pathogen challenges (e.g., Tully and Franke 1935; Whiteley and Yuill 1989; Larsen *et al.* 1997; Wang *et al.* 1997).

In addition to weakening the immune defenses of listed species such as the brown pelican, excessive environmental selenium can also trigger pathogen and toxin challenges that would not otherwise have occurred. For example, a red tide flagellate (*Chattonella verruculosa)* which has caused the mortality of fish such as yellowtail, amberjack, red and black sea bream, has recently been discovered to require above-normal exposure to selenium (Imai *et al.* 1996). Only when selenium extracted from contaminated sediments is added to growth media can *C. verruculosa* sustain rapid growth (i.e., toxic blooms). The level of contamination required to sustain rapid growth is only about 2-times normal background. Clearly, the potential effects of selenium-mediated algal toxins must be considered when evaluating potential hazards associated with selenium criteria. The two episodes involving massive eared-grebe die-offs illustrate how quickly algal toxins can remove 10 percent or more of the entire continental population of a species. Selenium-mediated algal toxins should probably be viewed as a serious potential threat to any endangered species that could have major portions of its extant population exposed. The CTR-proposed criterion of 5 µg/L, which is more than 10-times the normal background concentration of waterborne selenium (e.g., Maier and Knight 1994), would almost always be associated with more than 2-times normal sediment selenium and therefore could facilitate toxic algal blooms.

The case of botulism that killed more than 1,400 brown pelicans at California's Salton Sea was a very unusual case of botulism that was mediated by a bacterial epizootic among fish (USDI-FWS 1997c). This bacterially-mediated pathway for an avian botulism epizootic had never been encountered before. Fish in the Salton Sea contain substantially elevated tissue selenium (e.g., Saiki 1990) which very plausibly leaves them immune impaired and hypersensitive to the *Vibrio* bacterial attacks that facilitated the botulism outbreak.

California Clapper Rail: The extant range of the California clapper rail is restricted to marshes of the San Francisco Bay Estuary, an aquatic system already receiving substantial selenium input from agricultural and industrial sources (Pease *et al.* 1992). California clapper rails feed almost exclusively on benthic invertebrates, a well-documented pathway for bioaccumulation of selenium (see review by Pease *et al.* 1992). Total inflows of water to the San Francisco Bay Estuary average less than 5 µg/L (ppb) selenium (e.g., inflows diverted to the Central Valley Project and State Water Project canals usually average about 1 µg/L (ppb) selenium). The Regional Monitoring Program for 1997 (SFEI, 1999) reported total selenium concentrations ranged from 0.03 µg/L (ppb) to 2.20 µg/L (ppb) with highest concentrations found in the south bay. Lonzarich *et al.* (1992) reported that eggs of California clapper rails collected from the north bay in 1987 contained up to 7.4 µg/g selenium. Water data from this time and location are not available. The *in ovo* threshold for selenium exposure that causes toxic effects on embryos of California clapper rails is unknown. For another benthic-foraging marsh bird, the black-necked stilt, the *in ovo* threshold for embryotoxicity is 6 µg/g selenium (Skorupa 1998). More recent investigations of fail to hatch California clapper rail eggs in the south bay in 1992 and the north bay in 1998 have not duplicated the higher selenium results of Lonzarich *et al.* and maximum egg selenium concentrations have not exceeded 3.2 µg/g (dw)(FWS unpublished data).

It has recently been demonstrated for mallard ducks that interactive effects of selenium and mercury can be super-toxic with regard to embryotoxic effects (Heinz and Hoffman 1998). Lonzarich *et al.* (1992) also reported potentially embryotoxic concentrations of mercury in eggs of California clapper rails. Abnormally high numbers of nonviable eggs, 13.7-22.9 percent, have also been reported for the California clapper rail (Schwarzbach 1994). Since the main avenue of impacts from selenium and mercury alone, and interactively, would be manifested as reproductive impairment (especially inviable eggs), it strongly appears that populations of the California clapper rail could not tolerate the increased selenium loading to the San Francisco Bay Estuary that would be allowable under a CTR-proposed criterion of 5 µg/L (ppb). Based, in part, on the data for California clapper rails, staff technical reports prepared for the San Francisco Bay Regional Water Quality Control Board recommend decreasing current selenium loading to the estuary by 50 percent or more (Taylor *et al.* 1992, 1993). By comparison, the CTR-proposed selenium criteria would possibly accommodate increases in selenium loading to the bay or locally elevated selenium in effluent dominated tributaries. If selenium concentrations or selenium loads were increased in San Francisco Bay, clapper rail egg selenium would be expected to increase. The rail is particularly vulnerable to any locally elevated effluent concentrations of selenium as the rail generally occupies small home ranges of only a few acres

California Least Tern: As a piscivorous bird, much of the discussion provided above for the bald eagle regarding the inadequacy of the CTR-proposed selenium criteria may also apply to the California least tern. Consequently, until species-specific data are collected or species-specific modeling is conducted for the California least tern, a selenium criterion on the order of 1.4 µg/L (ppb) (generic piscivorous bird model; Lillebo *et al.* 1988) to 1.9 µg/L (ppb) (Bald eagle model; Peterson and Nebeker 1992) must be viewed as the applicable guidance for protection of California least terns from selenium poisoning. The CTR-proposed criterion of 5 µg/L (ppb) must

therefore be viewed as unprotective of California least terns.

Selenium analyses of least tern eggs collected from San Francisco Bay and San Diego Bay are reported by Hothem and Zador (1995). In San Francisco Bay the eggs contained up to 3.1 µg/g selenium and in San Diego Bay the eggs contained up to 2.9 µg/g selenium. Neither of those maximum values exceed currently recognized thresholds for avian embryotoxicity (for selenium as a solitary stressor). However, both sets of eggs also exhibited elevated concentrations of mercury which raises the possibility of super-toxic interaction effects as demonstrated for mallards by Heinz and Hoffman (1998). Waterborne concentrations of selenium in the San Francisco Bay Estuary are currently well below 5 µg/L (ppb) (e.g., <1 µg/L (ppb); Pease *et al.* 1992).

Eggs of the Interior least tern (*Sterna antillarum athalassos*) collected from the Missouri River system in the central United States have contained as much as 11-12 µg/g selenium (Ruelle 1993; Allen and Blackford 1997). Allen and Blackford (1997) reported that Least Tern nesting success from 1992-1994 at most locations in the study area was not sufficient to ensure survival of the studied populations. They also concluded that although flooding and predation likely are the major cause of the low recruitment, the results of their study "indicate that selenium and mercury may contribute to low reproduction." Neither Ruelle (1993) nor Allen and Blackford (1997) reported what the waterborne selenium levels were at their study sites. Other authors have reported selenium concentrations averaging about 2-4 µg/L (ppb) for major tributaries of the Missouri River system (North Platte River, See *et al.* 1992; James River, USDI-FWS 1989).

Results from studies of the Interior least tern suggest that selenium concentrations in California least tern eggs would substantively exceed the 6 µg/g threshold for embryotoxicity established for black-necked stilts (Skorupa 1998) if selenium concentrations were permitted to rise to a 5 µg/L (ppb) concentration In combination with elevated mercury concentrations already noted for eggs of California least terns (Hothem and Zador 1995), significant reproductive impairment would be the expected outcome.

Light-footed Clapper Rail: The Service is not aware of any existing data for selenium concentrations in eggs of light-footed clapper rails, or for any other tissues. The Service is also not aware of any studies characterizing the selenium profile of marshes currently supporting populations of light-footed clapper rails. Insufficient information is available to determine the likelihood of the CTR-proposed selenium criterion of 5 µg/L (ppb) being fully met within marshes crucial to survival and recovery of the light-footed clapper rail.

Because light-footed clapper rails have declined to just a few remnant populations vulnerable to rapid extirpation (Baron and Jorgensen 1994), are relatively sedentary nonmigratory residents prone to maximum exposure to localized contamination of a marsh, and are linked to a benthic foodchain that would be very efficient at bioaccumulating selenium, a worst-case scenario for potential impacts associated with a proposed 5 µg/L (ppb) selenium criterion must be assumed. Based on data for the California clapper rail and the Yuma clapper rail (summarized in this final biological opinion) a worst-case scenario of environmental selenium contamination up to the limits

allowed by the proposed CTR criteria would include *in ovo* exposure to selenium substantially above best estimates of the embryotoxic threshold. Particularly if elevated levels of environmental selenium were established in the presence of elevated levels of mercury, selenium-induced or selenium/mercury interactively-induced reproductive failure could occur.

Marbled Murrelet: During the breeding season marbled murrelets forage in nearshore environments including bays and estuaries on small fish and euphasid shrimp. They have also been known to forage to a minor degree on salmonid fry in freshwater environments. As a piscivorous bird, much of the discussion provided above for the bald eagle regarding the inadequacy of the CTR-proposed selenium criterion may also apply to the marbled murrelet.

Adverse impacts from increased permissible concentrations of contaminants as proposed in the CTR to prey species such as the Pacific sardine, herring, topsmelt, and northern anchovies, has the potential to significantly reduce long-term reproductive success of marbled murrelets (USDI-FWS, 1997b). Adverse effects to prey species spawning and nursery habitats have the potential to impair population size and reduce recruitment throughout their range in California. The vulnerability of marbled murrelet populations in conservation zones 5 and 6, coupled with elevated concentrations of contaminants in spawning and nursery areas for murrelet prey species increase the risk of bioaccumulation of mercury and selenium. The synergistic effects of these contaminants pose a significant threat to marbled murrelet reproduction throughout conservation zones 5 and 6 and to a lesser degree in conservation zone 4.

Consequently, until species-specific data are collected or species-specific modeling is conducted for the marbled murrelet, a selenium criterion on the order of 1.4 µg/L (ppb) (generic piscivorous bird model; Lillebo *et al*. 1988) to 1.9 µg/L (ppb) (bald eagle model; Peterson and Nebeker 1992) must be viewed as the applicable guidance for protection of marbled murrelets. Foraging in environments with between 2 and 5 µg/L (ppb) selenium during the breeding season would likely present a reproductive hazard to the murrelet. The Services therefore conclude that the CTR-proposed criterion of 5 µg/L (ppb) must be viewed as unprotective of marbled murrelets foraging in enclosed bays and estuaries in the State of California.

Western Snowy Plover: Interior populations of the western snowy plover have been studied at breeding sites averaging about 5 µg/L (ppb) waterborne selenium in California's Tulare Lake Basin (Skorupa *et al*. unpubl. data). At those sites, eggs averaged about 9 µg/g selenium. That exceeds the 6 µg/g threshold for embryotoxicity among black-necked stilts, but species-specific data for snowy plover eggs containing a wide range of selenium concentrations (egg selenium from 2-50 µg/g) suggest that snowy plovers are less sensitive to selenium exposure than black-necked stilts (Skorupa *et al*. unpubl. data; Page *et al*. 1995; Washington Dept. of Fish and Wildlife 1995). Western snowy plovers appear to be about as tolerant of selenium exposure as American avocets (*Recurvirostra americana*) (cf. Skorupa 1996; 1998) which suggests that they would not be at risk of reproductive impairment when nesting at sites with up to 5 µg/L (ppb) waterborne selenium. The study sites producing this data for interior-nesting snowy plovers were uniformly uncontaminated with mercury (Skorupa *et al*. unpubl. data).

Unless coastal populations would be exposed to significant selenium-mercury interaction effects (cf. Heinz and Hoffman 1998), the results documented for populations of interior-nesting snowy plovers are expected to apply to the listed Pacific Coast populations of the snowy plover. Therefore, the western snowy plover is considered not likely to be adversely affected by the CTR-proposed selenium criterion of 5 µg/L (ppb).

Yuma Clapper Rail: With a biological profile very similar to the California clapper rail, the Yuma clapper rail is similarly vulnerable to selenium bioaccumulation via a benthic foodchain pathway. For backwaters of the lower Colorado River system in California, Lonzarich *et al.* (1992) reported a mean selenium concentration of 12.5 µg/g selenium for eggs from two abandoned clutches of Yuma clapper rails. They also stated that this level of exposure was "..believed to be associated with low hatching success and embryo deformities..." (Lonzarich *et al.* 1992:151). A mean of 12.5 µg/g *in ovo* selenium substantively exceeds the 6 µg/g threshold for embryotoxicity rigorously established for another benthic-foraging species of marshbird, the black-necked stilt (Skorupa 1998). The source water for the Colorado River backwaters where these Yuma clapper rail eggs were sampled averages about 2 µg/L (ppb) selenium (e.g., Setmire and Schreder 1998). Clearly, if selenium in the source water increased to 5 µg/L (ppb) as would be allowable under the CTR-proposed selenium criterion, it could be expected that the selenium content of Yuma clapper rail eggs would very substantially exceed the best available estimate of the embryotoxic threshold point.

Agricultural drainage water in the Imperial Valley typically contains 2-10 µg/L (ppb) selenium (see review for Salton Sea in Skorupa 1998). When marshes in the Imperial Valley were supplied with agricultural drainwater in 1990, selenium concentrations in a sample of Yuma clapper rail eggs were as high as 7.8 µg/g (C. Roberts, pers. comm.). When the drainage water was replaced with water containing 2 µg/L (ppb) selenium, the concentrations of selenium measured in Yuma clapper rail foods (crayfish) were at safe levels (2.2 µg/g). The data from the Colorado River and from the Imperial Valley, the major extent of the Yuma clapper rail's geographic range, are consistent in indicating that a selenium criterion of 5 µg/L (ppb) would not be adequately protective.

Amphibians and Reptiles

Selenium is toxic to developing frog embryos and tadpoles (Browne and Dumont, 1979), however, testing of amphibians has been very limited. Browne and Dumont for example only tested sodium selenite and only in short term acute tests. Most field studies of selenium do not include amphibians and those that do generally report uninterpreted residues in frog liver. The Service is unaware of specific studies of amphibian egg residues and associated impacts to reproduction, however, it is likely that amphibian toxic response is similar to fish and birds where reproductive failure is associated with egg concentrations greater than 6 µg/g in birds and 10 µg/g in fish. It is also likely that aquatic food chain contamination by selenium would be the most significant pathway of exposure as would maternal transfer of organic selenium to the eggs. In the absence of selenium toxicity information the Service believes a fish risk model may be most appropriate for

assessing selenium hazard to amphibians such as the red-legged frog. This assessment may however be overly simplistic. Development of amphibians is unique among vertebrates in the occurrence of hormone mediated ontogenetic metamorphosis within the water column (Duellman and Trueb, 1986) and selenium is a notorious developmental toxin and growth inhibitor (Skorupa, 1998). Dietary selenium exposure of tadpoles may thus be another significant route of exposure affecting development. California red-legged frogs spend most of their lives in and near sheltered backwaters of ponds, marshes, springs, streams, and reservoirs. These types of environments are particularly vulnerable to selenium contamination of the food chain at low to medium level selenium contamination in water, should a selenium source to water exist. Red legged frogs are now reduced to about 30 percent of their historical range with most of the remaining population limited to coastal drainages. The cretaceous shales of the coast range of California provide a bulk source of selenium whose release to water bodies is accelerated by anthropogenic activities such as cattle grazing, and irrigation drainage. The Service therefore concludes that a criterion of 5 µg/L (ppb) may not be sufficiently protective for the red-legged frog.

Toxicity information on reptiles such as the giant garter snake are even more scanty than the amphibian literature. The Service is unaware of any such information. Endemic to wetlands in the Sacramento and San Joaquin Valleys, the giant garter snake inhabits marshes, sloughs, ponds, small lakes, low gradient streams, and other waterways and agricultural wetlands, such as irrigation and drainage canals and rice fields. Giant garter snakes feed on small fishes, tadpoles, and frogs (Fitch 1941, Hansen 1980, Hansen 1988). These foraging habits and habitat preference put the giant garter snake at risk of selenium exposure. The current day absence of the giant garter snake from extensive wetland areas (the Grasslands Water District) of the San Joaquin Valley, which for the last twenty years have received seleniferous irrigation drainage water, may be circumstantial evidence of a selenium effect on this top aquatic predator. In the absence of a species specific selenium toxicity model for the giant garter snake the Service would recommend using an avian risk model for selenium based on the close phylogenetic relationship of birds to reptiles (e.g., Romer 1966; Porter 1972:216; Storer et al. 1972:312). The Service concludes that a selenium criterion of 5 µg/L (ppb) would not adequately protect the giant garter snake.

Fish

A tremendous amount of research regarding toxic effects of selenium on fish has been conducted since the late 1970's. Recently, this body of research was reviewed and summarized by Lemly (1996b). Lemly reports that salmonids are very sensitive to selenium contamination and exhibit toxic symptoms even when tissue concentrations are quite low. Survival of juvenile rainbow trout (*Oncorhynchus mykiss*) was reduced when whole-body concentrations of selenium exceeded 5 µg/g (dry wt.). Smoltification and seawater migration among juvenile chinook salmon (*Oncorhynchus tshawytscha*) were impaired when whole-body tissue concentrations reached about 20 µg/g. However, mortality among larvae, a more sensitive life stage, occurred when concentrations exceeded 5 µg/g. Whole-body concentrations of selenium in juvenile striped bass (*Morone saxitilis*) collected from areas in California impacted by irrigation drainage ranged from 5 to 8 µg/g.

Summarizing studies of warm-water fish Lemly reports that growth was inhibited at whole-body tissue concentrations of 5 to 8 µg/g selenium or greater among juvenile and adult fathead minnows (*Pimephales promelas*). Several species of centrarchids (sunfish) exhibited physiologically important changes in blood parameters, tissue structure in major organs (ovary, kidney, liver, heart, gills), and organ weight-body weight relations when skeletal muscle tissue contained 8 to 36 µg/g selenium. Whole-body concentrations of only 4 to 6 µg/g were associated with mortality when juvenile bluegill (*Lepomis macrochirus*) were fed selenomethionine-spiked commercial diets in the laboratory. When bluegill eggs contained 12 to 55 µg/g selenium, transfer of the selenium to developing embryos during yolk-sac absorption resulted in edema, morphological deformities, and death prior to the swim-up stage. In a laboratory study of "winter stress syndrome" juvenile bluegill exposed to a diet containing 5.1 µg/g selenium and water containing 4.8 µg/L (ppb) selenium exhibited hematological changes and gill damage that reduced respiratory capacity while increasing respiratory demand and oxygen consumption. In combination with low water temperature (4 degrees centigrade) these effects caused reduced activity and feeding, depletion of 50 to 80 percent of body lipid, and significant mortality within 60 days. Winter stress syndrome resulted in the death of about one-third of exposed fish at whole-body concentrations of 5 to 8 µg/g selenium.

Based on Lemly's review of more than 100 papers, he recommended the following toxic effects thresholds for the overall health and reproductive vigor of freshwater and anadromous fish exposed to elevated concentrations of selenium: 4 µg/g whole body; 8 µg/g skinless fillets; 12 µg/g liver; and 10 µg/g ovary and eggs. He also recommended 3 µg/g as the toxic threshold for selenium in aquatic food-chain organisms consumed by fish. Lemly reported that when waterborne concentrations of inorganic selenium (the predominant form in aquatic environments) are in the 7- to 10-µg/L (ppb) range bioconcentration factors in phytoplankton are about 3,000. Consequently, he concluded that patterns and magnitudes of bioaccumulation are similar enough among various aquatic systems that a common number, 2 µg/L (ppb) (for filtered samples of water), could be given as a threshold for conditions "highly hazardous to the health and long-term survival of fish".

Recently, Hamilton (1998) reviewed the demonstrated and potential effects of selenium on six species of endangered fish in the Colorado River basin, including the humpback chub (*Gila cypha*), Colorado squawfish (*Ptychocheilus lucius*), bonytail chub (*Gila elegans*), razorback sucker (*Xyrauchen texanus*), flannelmouth sucker (*Catostomus latipinnis*), and roundtail chub (*Gila robusta*). Hamilton presents historical data supporting a hypothesis that long-term selenium contamination of the lower Colorado River basin may have been one of the factors contributing to the disappearance of endangered fish in the early 1930's. Contemporary issues of concern included the unusually high incidence of abnormal lesions on fish in the San Juan River, especially flannelmouth sucker, attributed to pathogens requiring inducement by stressors such as high contaminant concentrations or poor body condition; and concentrations of selenium in fish eggs as high as 28 µg/g in razorback sucker from the Green River and as high as 73 µg/g in eggs of rainbow trout collected from the mainstem Colorado River between Glen Canyon Dam and Lee's Ferry. In controlled studies of larval razorback suckers fed food organisms collected from

the wild, Hamilton found 2.3 µg/g or more of selenium in the diet to be sufficient to cause reduced survival. In an enclosure study where razorback suckers were held in selenium-contaminated aquatic environments (Adobe Creek, 9-90 µg/L (ppb) selenium, and North Roadside Pond of Ouray National Wildlife Refuge, 40 µg/L (ppb) selenium) for 9 months, muscle plugs contained 17 and 12 µg/g selenium respectively and eggs contained 44 and 38 µg/g selenium. Finally, Hamilton stressed that consideration of selenium effects was an important component of recovery planning for the Colorado River basin endangered endemics.

Selenium effects on Delta Fishes: In November of 1996 the Service issued an approved Recovery Plan for the Sacramento/San Joaquin Delta Native Fishes (USDI-FWS 1996c). The plan addressed recovery requirements for eight species of fish native to the Delta including one species currently listed as threatened, the Delta Smelt (*Hypomesus transpacificus*), and the proposed threatened Sacramento Splittail (*Spirinchus thaleichthys*). Other species addressed by the plan are Longfin Smelt (*Spirinchus thaleichthys*), Green Sturgeon (*Acipenser medirostris*), the Sacramento Spring-run chinook salmon (*Oncorhynchus tshawytscha*), which has been petitioned for listing as endangered, the Sacramento Late Fall-run chinook salmon (*Oncorhynchus tshawytscha*), the San Joaquin Fall-run chinook salmon (*Oncorhynchus tshawytscha*), and the extirpated Sacramento Perch (*Archoplites interruptus*). The Sacramento-San Joaquin River Delta and San Francisco Bay estuary are subject to elevated levels of environmental selenium, and the introduction of high levels of contaminants (including selenium) is cited in the Recovery Plan as one of the more recent potential factors affecting Delta fishes.

Lillebo *et al.* (1988) calculated that a selenium criterion of 0.9 µg/L (ppb) waterborne selenium was necessary to adequately protect fish associated with the San Joaquin River system, including the southern Delta. The CTR-proposed selenium criterion of 5 µg/L (ppb) substantially exceeds the criterion calculated by Lillebo *et al.* (1988). The Recovery Plan states that Delta Smelt are ecologically similar to larval and juvenile Striped Bass (*Morone saxitilis*). Saiki and Palawski (1990) sampled juvenile striped bass in the San Joaquin River system including three sites in the San Francisco Bay estuary. Striped Bass from the estuary contained up to 3.3 µg/g whole-body selenium, a value just below Lemly's 4 µg/g toxicity threshold, even though waterborne selenium typically averages <1 µg/L (ppb) and has been measured no higher than 2.7 µg/L (ppb) within the estuary (Pease *et al.* 1992). Striped Bass collected from Mud Slough in 1986, when the annual median selenium concentration in water was 8 µg/L (ppb) (Steensen *et al.* 1997), contained up to 7.9 µg/g whole-body selenium and averaged 6.9 µg/g whole-body selenium. Saiki and Palawski's results suggest that water fully meeting the CTR-proposed 5 µg/L (ppb) criterion could result in Delta Smelt with whole-body selenium concentrations exceeding the toxic threshold of 4 µg/g. Delta Smelt spawning sites are almost entirely restricted to the north-Delta channels associated with the selenium-normal Sacramento River and are nearly absent from the south-Delta channels associated with the selenium-contaminated San Joaquin River (USDI-FWS 1996c).

White Sturgeon (*Acipenser transmontanus*), a representative surrogate species for the Green sturgeon, have been the subject of detailed studies within the San Francisco Bay estuary (e.g., Kohlhorst *et al.* 1991). White Sturgeon are long-lived, large-bodied, and demersal (bottom-

dwelling) fish. For most species of sturgeon, females require several years for eggs to mature between spawnings (Conte *et al*. 1988). White Sturgeon in the San Francisco Bay estuary congregate in Suisun and San Pablo Bays where they remain year-round except for a small fraction of the population that moves up the Sacramento River, and to a lesser extent the San Joaquin River, to spawn in late winter and early spring (Kohlhorst *et al*. 1991). Thus, many individuals of this species remain year-round in San Pablo Bay, the part of the San Francisco Bay estuary with the highest selenium concentrations (up to 2.7 µg/L (ppb)). Kroll and Doroshov (1991) report that developing ovaries of White Sturgeon from San Francisco Bay contained as much as 71.8 µg/g selenium, or 7-times over the threshold for reproductive toxicity (Lemly 1996a, 1996b) of 10 µg/g. Sampling of Pallid Sturgeon (*Scaphirhynchus albus*) in the Missouri River system suggests that normal selenium levels in sturgeon eggs are 2-3 µg/g (Ruelle and Keenlyne 1993) as has been found for many other fish species (see review in Skorupa *et al*. 1996 and in USDI-BOR/FWS/GS/BIA 1998). Thus, White Sturgeon in the San Francisco Bay estuary are producing eggs with as much as 35-times normal selenium content. Based on studies regarding toxicity response functions for avian and fish eggs (e.g., Lemly 1996a,b; Skorupa *et al*. 1996; USDI-BOR/FWS/GS/BIA 1998) it is highly probable that these fish are severely reproductively impaired due to selenium exposure. For example, bluegill embryos resulting from ovaries containing 38.6 µg/g selenium exhibited 65 percent mortality (Gillespie and Bauman 1986).

It is quite plausible that a waterborne concentration of 5 µg/L (ppb) selenium in the San Francisco Bay estuary, as would be allowable for effluent-dominated waters under the CTR-proposed selenium criterion, would result in complete reproductive collapse of sturgeon populations as well as elevated tissue concentrations in Delta Smelt above the 4 µg/g whole-body toxicity threshold.

Selenium effects to Salmonids: Salmonid species considered in this opinion are coho salmon, including Central California Coast and Southern Oregon/Northern California Coast ESUs; chinook salmon, including the Central Valley Spring-Run, the California Coastal, and the Sacramento River Winter-Run ESUs; steelhead trout, including the Central Valley, the Southern California, the South-Central California Coast, the Central California Coast, and Northern California ESUs; Lahontan cutthroat trout; Paiute cutthroat trout, and Little Kern golden trout. Salmonids are considered sensitive to selenium contamination (see review in Lemly 1996a,b). Depending on the form of selenium and the life-stage of fish considered, waterborne concentrations of selenium less than the CTR-proposed 5 µg/L (ppb) concentration can have direct toxic impacts on salmonids (Hodson *et al*. 1980; Moore *et al*. 1990). Hodson *et al*. reported that rainbow trout (*O. mykiss*) eggs respond physiologically (reduced median time to hatch) at selenium (as selenite) concentrations above 4.3 µg/L (ppb).

However, the most dangerous exposure pathway for salmonids, as with other fish, is via dietary bioaccumulation of selenium. As little as 3.2 µg/g selenium in the diet was sufficient to adversely affect early life stages of chinook salmon under controlled conditions (Hamilton *et al*. 1989; 1990). Based on a bioaccumulation factor for dry weight concentrations of selenium in aquatic invertebrates (compared to water) of 1,800 (Pease *et al*. 1992), a concentration of as little as 1.8 µg/L (ppb) selenium could result in salmonid foods averaging more than 3.2 µg/g selenium. That

water concentration is already exceeded at times in San Pablo Bay (Pease *et al*. 1992), in the San Joaquin River (Steensen et al. 1997), in the Santa Ynez River (Westcot et al. 1990), in the Pajaro River (Westcot et al. 1990), and in the Salinas River (Westcot et al. 1990). If California's water bodies that currently support salmonid populations were allowed to have concentrations which meet the CTR-proposed selenium criterion of 5 µg/L (ppb), salmonid food organisms would be expected to contain an average of about 9 µg/g selenium (based on a bioaccumulation factor of 1,800). That value exceeds even the 6.5 µg/g dietary toxicity threshold for older life stages of chinook salmon in brackish-water (Hamilton et al. 1989; 1990). Hamilton et al. (1990) also found that dietary exposure of swim-up chinook salmon to 9.6 µg/g selenium resulted in reduced survival after 90 days. The Services thus conclude that currently available data for salmonids do not support the CTR-proposed selenium criterion of 5 µg/L (ppb) as adequately protective of salmonids.

Desert Pupfish: Specific data exist to support a conclusion that the desert pupfish would be unprotected by a chronic selenium criterion of 5 µg/L (ppb). Setmire and Schroeder (1998) report on a field study of sailfin mollies in the Salton Sea area of California. The mollies were chosen as surrogate species in order to assess contaminant threats to the co-occurring endangered desert pupfish. Mollies and pupfish were simultaneously collected from one site and found to contain virtually identical whole-body selenium concentrations (Bennett 1997), which verified the utility of mollies as a surrogate indicator of pupfish exposure. During 1994, mollies were collected from 13 agricultural drains. For 10 of the 13 drains, whole-body selenium concentrations were in the range of 3 to 6 µg/g, a level designated by a panel of selenium researchers as "of concern" for warmwater fishes (USDI-BOR 1993; also see Gober 1994; CAST 1994; Ohlendorf 1996). Two of the other three drains that were sampled yielded mollies averaging >6 µg/g, a level designated by the panel of researchers as exceeding the toxic threshold for warmwater fishes. Unfortunately, contemporaneous measures of waterborne selenium in the sampled drains were not obtained for comparison to the mollie tissue data.

An inquiry with California's Colorado River Basin Regional Water Quality Control Board yielded file data on waterborne selenium for one of the 13 drains sampled for mollies in 1994; however the file data is for water samples collected in 1996 (R. Lukens, Regional Water Board, pers. comm.). Ten monthly (March to December, 1996) measures of waterborne selenium in the Trifolium 12 drain averaged 4.96 µg/L (ppb). Sailfin mollies collected from Trifolium 12 drain in 1994 averaged 3.6 µg/g whole-body selenium, with a maximum of 3.8 µg/g (n=3). If the concentrations of selenium in the drain were roughly the same in 1994 as in 1996, then the CTR-proposed selenium criterion of 5 µg/L (ppb) would be associated with expected pupfish tissue concentrations of selenium at the "level of concern". As discussed in the species effect account for brown pelicans, borderline exposures for direct toxic effects may be particularly hazardous at the Salton Sea because of the recent record of diverse and frequent epizootic events documented for fish and birds at the Sea. It is well established for birds that selenium-induced immune dysfunction occurs at exposure levels below those required for direct selenium-poisoning. Until comparable studies are completed for fish, the safest default assumption is that the results for selenium-induced immune dysfunction documented for birds may also apply to fish.

The CTR-proposed selenium criterion of 5 µg/L (ppb) does not provide the margin of safety necessary to confidently conclude that the criterion would adequately safeguard survival and recovery of desert pupfish. It is also clear that selenium routes of exposure exist for the desert pupfish which put them at risk. The Services therefore conclude that the CTR-proposed selenium chronic criterion for selenium of 5 µg/L (ppb) does not adequately protect the desert pupfish.

Given the above effects analysis, the Services, in our draft opinion dated April 10, 1998 concluded that the selenium criteria as described by EPA in their August 1997 proposed CTR would be insufficiently protective. Implementation of these selenium criteria without future modification could jeopardize the continued existence of the following species: marbled murrelet, California clapper rail, California least tern, light-footed clapper rail, Yuma clapper rail, bonytail chub, coho salmon (California ESUs), delta smelt, desert pupfish, steelhead (California ESUs) Razorback sucker, Chinook salmon (California ESUs), Sacramento splittail, Giant garter snake, and California red-legged frog. It was the Services' opinion that a criterion of 2 µg/L or less would be necessary for protection of these species, that the proposed speciation based acute criterion should not be promulgated and that a selenium criteria revision which considered the bioaccumulative nature and long term persistence of selenium in aquatic sediments and food chains was necessary in the development of new criteria and a site specific guidance for criteria modification.

EPA modifications addressing the Services' April 9, 1999 draft Reasonable and Prudent Alternatives for selenium:

The above effect analysis considers the draft CTR as originally proposed in August of 1997.

EPA has agreed by letter dated December 16, 1999 to modify its action for selenium criteria per the following to avoid jeopardizing listed species.

A. *EPA will reserve (not promulgate) the proposed acute aquatic life criterion for selenium in the final CTR.*

B. *EPA will revise its recommended 304(a) acute and chronic aquatic life criteria for selenium by January 2002. EPA will propose revised acute and chronic aquatic life criteria for selenium in California by January of 2003. EPA will work in close cooperation with the Services to evaluate the degree of protection afforded to listed species by the revisions to these criteria. EPA will solicit public comment on the proposed criteria as part of its rulemaking process, and will take into account all available information, including the information contained in the Services' opinion, to ensure that the revised criteria will adequately protect federally listed species. If the revised criteria are less stringent than those proposed by the Services in the opinion, EPA will provide the Services with a biological evaluation/assessment on the revised criteria by the time of the proposal to allow the Services to complete a biological opinion on the proposed selenium criteria before promulgating final criteria. EPA will*

provide the Services with updates regarding the status of EPA's revision of the criterion and any draft biological evaluation/assessment associated with the revision. EPA will promulgate final criteria as soon as possible, but no later than 18 months, after proposal. EPA will continue to consult, under section 7 of ESA, with the Services on revisions to water quality standards contained in Basin Plans, submitted to EPA under CWA section 303, and affecting waters of California containing federally listed species and/or their habitats. EPA will annually submit to the Services a list of NPDES permits due for review to allow the Services to identify any potential for adverse effects on listed species and/or their habitats. EPA will coordinate with the Services on any permits that the Services identify as having potential for adverse effects on listed species and/or their habitat in accordance with procedures described in the draft MOA published in the Federal Register at 64 Fed. Reg. 2755 (January 15, 1999) or any modifications to those procedures agreed to in a finalized MOA.

C. EPA will utilize existing information to identify water bodies impaired by selenium in the State of California. Impaired is defined as water bodies for which fish or waterfowl consumption advisories exist or where water quality criteria necessary to protect federally listed species are not met. Pursuant to Section 303(d) of the CWA, EPA will work, in cooperation with the Services, and the State of California to promote and develop strategies to identify sources of selenium contamination to the impaired water bodies where federally listed species exist, and use existing authorities and resources to identify, promote, and implement measures to reduce selenium loading into their habitat.

Services' assumptions regarding EPA's modifications for removing jeopardy.

The Services assume the following:

Contaminant threats to listed species can be reduced through application of appropriately protective water quality criteria to the water bodies occupied by listed species.

The presumptive adverse effect threshold for identifying effects to listed species, is either the exceedance of the criteria proposed in this opinion to protect listed species, or demonstrated effects below those proposed criteria concentrations for the priority pollutant under consideration.

The adjustments of criteria as proposed in the CTR by EPA for water bodies occupied by species considered in this opinion will be consistent with the effects analysis in this biological opinion unless new information is developed by EPA.

EPA adjustments of criteria will occur within agreed upon time frames.

The future adjustment of the selenium criteria will consider the bioaccumulative nature of

selenium in aquatic systems, not just the waterborne toxicity and will result in a lowering of the criteria. Thus listed fish and wildlife species which are aquatic system foragers will be protected by the future criteria and the procedures for site specific adjustments.

The reservation of the acute aquatic life criterion for selenium will result in the criterion being withheld from use for regulation by the State and Regional boards.

Mercury

Assessment of Adequacy of Proposed Mercury Criteria to protect listed species

Aquatic Life Criteria for Mercury

The EPA has proposed an acute aquatic life criterion (criterion maximum concentration or CMC) for mercury of 1,400 ng/L and a chronic aquatic life criterion (criterion continuous concentration or CCC) of 770 ng/L. These criteria are based upon dissolved concentrations. EPA's proposed mercury criteria for aquatic life are based on the assumed waterborne toxicity of dissolved forms of mercury to aquatic organisms that exclusively live within the water column. The Services believe the proposed CTR aquatic life criteria for mercury will not protect listed fish from either dietary toxicity or maternal transfer of methylmercury to young. Promulgation of a dissolved mercury criteria also fails to consider the effects upon biota of particulate methylmercury and particulate inorganic mercury. Regulation of mercury on a dissolved basis only for aquatic life ignores the role of particulate mercury in the cycling of mercury in aquatic ecosystems and the need to consider the dietary pathway for mercury accumulation in aquatic life.

The aquatic life mercury criteria of 770 ng/L(chronic) and 1,400 ng/L (acute) are so high as to effectively be without value for controlling mercury in even the most severely mercury-impaired California water bodies. Concentrations above the chronic criterion concentration in the dissolved form are virtually unmeasured in the California environment, even though those environments contain numerous water bodies with direct mercury discharges. In a broad survey of mercury in freshwater systems in California and other areas, Gill and Bruland (1990) failed to locate any water bodies containing levels of mercury above or approaching these dissolved criteria although many of these same water bodies were mercury impaired due to elevated mercury concentrations in fish.

Two California examples illustrate why the chronic and acute criteria for mercury are unreasonably high with no potential to impact or control mercury concentrations. Walker Creek is potential habitat for both steelhead and the California red-legged frog and discharges into Tomales Bay. The Gambonini mine, an abandoned mercury mine, produces concentrations of total mercury in unfiltered water from Walker Creek as great as 100,000 ng/L, yet dissolved concentrations in the creek only range from 20 to 100 ng/L (Whyte 1998). These concentrations are of great concern as evidenced by Regional Board activity to cleanup and restore the mine site, but obviously well below EPA's proposed chronic aquatic life criterion of 770 ng/L. The aquatic

life criteria of EPA would likely be controlling for Walker Creek as fish consumption from the creek is not a beneficial use and Walker Creek lacks a MUN designation (use for municipal drinking water purposes). Long *et al*. (1990) unexpectedly found toxicity to three species in sediments of Tomales Bay (their control site) and found the sediments of Tomales Bay devoid of the more sensitive crustaceans corroborating toxicity test results. This toxicity was best explained by the mercury as it was the only toxicant present at elevated concentrations.

Davis Creek Reservoir in the Cache Creek watershed is another example. This site is highly contaminated by mercury. This reservoir is also potential foraging habitat for the bald eagle as up to 60 eagles winter in this drainage. Davis Creek Reservoir has dissolved organo-mercury concentrations of 60 picomoles (12 ng/L) associated with a total dissolved mercury concentration of 16 ng/L and total unfiltered mercury concentrations of 26 to 32 ng/L. These concentrations of mercury in water were associated with fish tissue concentrations of 2.5 µg/g (ppm) wet weight (Gill and Bruland 1990). The fish mercury concentrations present significant risk to any foraging eagles. The proposed chronic aquatic life criterion for mercury at this reservoir, which probably is not covered by human health criteria as it is a water supply for processing gold ores, are an order of magnitude above all concentrations observed at this site.

Human Health Mercury Criterion (for Protection of Fish and Wildlife)

Since the aquatic life criteria clearly are not protective of fish and wildlife, the Services have evaluated whether the lower human health criterion of 50 ng/L would be protective. The Services find that the human health criterion for mercury will not protect listed fish or wildlife species. The EPA's biological evaluation (BE) (EPA 1997a) states that the human health criterion of 50 ng/L (total mercury), will offer protection of aquatic life in the water column and to non-aquatic piscivorous birds and mammals. Footnote a, page 42204 of the August 5, 1997, Federal Register (EPA 1997c) notes that for mercury "The fish tissue bioconcentration factor (BCF) from the 1980 documents was retained..." Unfortunately these bioconcentration factors were derived prior to modern developments in analytical chemistry that permit more accurate determination of concentrations of mercury in water. The resulting 1980 bioconcentration factor of 7,342.6 used to derive the proposed mercury criterion is neither appropriate, accurate, or reflective of real world environmental mercury concentrations in water. As a result of improvements after 1988 in water chemistry for mercury, it is now clear that mercury concentrations are far lower than was thought in 1980, and consequently bioconcentration factors and bioaccumulation factors have been revised and are now known to be far higher than those used by EPA in the CTR. This scientific information is well known and has been available for a decade (EPA 1997b; Bloom 1989; Bloom and Fitzgerald 1988). The Services therefore find the statement within the biological evaluation for the CTR that "the human health criteria for mercury will protect listed wildlife" is not supported by the best scientifically and commercially available data. In addition the Services also anticipate the criterion will not be sufficiently protective of the potential for maternal transfer of harmful concentrations of mercury to vertebrate eggs and embryos.

EPA indicated during informal consultation that the human health criterion for mercury may be

changed in the near future. Should an appropriate bioaccumulation factor for mercury be applied at some future date to develop a human health criterion either in water or in fish tissue, it is not necessarily clear that such a criterion designed for protection of human health alone would also afford adequate protection to listed species. Because fish and wildlife typically have more restricted diets than humans, they are more susceptible to local contamination. Wildlife, particularly piscivorous wildlife, are often at greatest risk from mercury exposure within any ecosystem (EPA 1997b). Even with appropriate bioaccumulation factors for evaluating human fish consumption, the use of humans as the surrogate species to represent the bioaccumulation hazards presented to wildlife is not scientifically supported. "Fish-eating wildlife are more vulnerable to the adverse effects of mercury than are humans for two reasons: (1) fish compose a higher proportion of their diet: and (2) wildlife are more dependent on their reflexes to survive." (A. Kuzmack, EPA, pers comm., February 17, 1998).

Hazards to Species: Toxicity and Bioaccumulation

Toxicity

Mercury is a trace element with no known essential biological function. Mercury in environmental waters can exist in many forms including elemental form (Hg^0), dissolved and particulate ionic forms, and dissolved and particulate methylmercury (Gill and Bruland 1990; Vandal et al 1991; Mason and Fitzgerald 1993). Methylmercury may be formed either in the water column or in sediment.

Methylmercury is the most toxic and the most bioaccumulated form of mercury. Intestinal absorption of inorganic mercury is limited to a few percent while absorption of methyl mercury is nearly complete (Scheuhammer 1987). Inorganic mercury appears to have the greatest effect upon the kidneys, while methylmercury is a potent embryo and nervous system toxicant. Methylmercury readily penetrates the blood brain barrier, produces brain lesions, spinal cord degeneration, and central nervous system dysfunctions. The proportion of total mercury which is found as methylmercury in biota increases with trophic level approaching 100% at trophic levels 3 and 4. Methylmercury is biomagnified between trophic levels in aquatic systems and in proportion to its supply in water (Wattras and Bloom, 1992). It is appropriate therefore to focus attention on the toxicity of methylmercury, particularly in higher trophic level organisms (Nichols et al., 1999).

Fish: In the 1995 update to Water Quality Criteria Documents for Mercury, EPA stated that the estimated chronic value for effects to coho salmon was 370 ng/L and 420 ng/L for rainbow trout. EPA further explicitly acknowledged that the CCC of 908 ng/l (the CCC in favor as of 1995) might not adequately protect these species (EPA 1995b). In the subsequent CTR, EPA has reduced the proposed CCC for mercury to 770 ng/L. However, this revised number also remains unprotective for federally listed salmonid species. For example, in flow through bioassays, fertilized eggs of rainbow trout suffered 100 percent mortality after 8 day exposures to 100 ng/L concentrations of inorganic mercury (Birge *et al.* 1979). In a review of mercury toxicity to fish,

Wiener and Spry (1996) noted direct adverse effects in a variety of fish species on behavior, growth, histology, reproduction, development and survival of fish at concentrations well below the proposed chronic criterion. Fish species tested with adverse effects below criteria concentrations include trout and fathead minnows.

Amphibians and Reptiles: Reptiles and amphibians remain the least studied vertebrates for mercury toxicity. Amphibian eggs and embryos may be the most vulnerable to direct waterborne concentrations. A dose of 50 µg/L applied to the embryos of the frog (*Xenopis laevis*) reduced survival by 50 percent after 4 days of treatment, and to 0 percent after 7 days. Surviving embryos showed disruption of morphogenesis, neurophysiology, and neuroimmune regulation (Ide et al, 1995). Rao and Madhyastha (1987) reported that the LC_{50} (the lethal concentration in water that kills 50 percent of the test organisms) of mercuric chloride to the tadpoles of (*Microhyla ornata*) ranged from 2.04 mg/L (24 hour) to 1.12 mg/L (96 hour). In leopard frog (*Rana pipiens)* embryos methylmercury concentrations of 40 µg/L and above were lethal (Dial 1976). Adverse affects were seen at concentrations as low as 10 µg/L. While these concentrations are well above the current criteria, they are also acute exposures of four to five days exposure and reflect no maternal transfer of methylmercury. Chronic studies in frogs of the effects of mercury contamination are generally lacking. The Service was not able to locate any published acute or chronic studies of mercury in snakes.

Birds: Symptoms of acute methylmercury poisoning in birds include reduced food intake leading to weight loss, progressive weakness in wings and legs, difficulty flying, walking, and standing, and an inability to coordinate muscle movements (Scheuhammer 1987). In addition to well-identified acute effects of mercury at high concentrations, there are also significant adverse effects at lower tissue-mercury concentrations representing chronic mercury exposures. Embryological exposure may possibly lead to impaired hearing, or altered behavior (Heinz 1979). Impaired or tunnel vision has been demonstrated in other adult vertebrate species (humans, and monkeys) (Wolfe *et al*. 1998). These sensory deficits could lead to reduced ability to locate and catch prey for the bald eagle or least tern, to impaired ability to find a mate through auditory clues in the clapper rail and an impaired ability to detect and escape predators in all species. In great white herons liver-mercury contamination > 6 µg/g correlated with mortality from chronic diseases (Sundloff *et al*. 1994).

Reproduction is one of the most sensitive toxicological responses, with effects occurring at very low dietary concentrations. Concentrations in the egg are typically most predictive of mercury risk to avian reproduction, but concentrations in liver have also been evaluated for predicting reproductive risk. The documented effects of mercury on reproduction range from embryo lethality to sublethal behavioral changes in juveniles at low dietary exposure. Reproductive effects in birds typically occur at only twenty percent of the dietary concentrations which produce lethal effects in adult birds (Scheuhammer 1991). Effects of mercury on reproduction are likely occurring in San Francisco Bay populations of birds due to concentrations of mercury observed in eggs including the least tern and the California clapper rail (Schwarzbach, et al, 1997).

Embryos of birds are extremely sensitive and vulnerable to relatively minute concentrations of mercury in the egg. Almost all of the mercury in bird eggs is thought to be methylmercury (Wolfe et al, 1998). Toxic effects of mercury in bird eggs have been documented by many investigators in both laboratory and field studies (Barr 1986; Birge *et al*. 1976; Fimreite 1971; Fimreite 1974; Heinz 1974; Heinz, 1975; Heinz 1979; Hoffman and Moore 1979; Finley and Stendell 1978; Tejning 1967; etc.). Fimreite estimated the threshold level in eggs for toxic effects to nest success in a field study of common terns to be between 1.0 and 3.6 µg/g. Heinz (1979) was able to examine more subtle behavioral effects in mallard ducklings fed methylmercury. Heinz fed ducks 0.5 µg/g mercury over 3 generations and found decreased reproductive success and altered behavior of ducklings. The Heinz study, remains the benchmark study which establishes the lowest observed adverse effect concentration in avian diet of 0.064 mg mercury/kg (body weight)/day (Sample *et al*. 1996). The mean mercury concentration in eggs associated with these observations was 0.86 µg/g fresh wet weight (fww). Fimreite in a 1971 mercury feeding study with ring-necked pheasants found significant reduction in hatchability associated with mercury levels between 0.5 and 1.5 µg/g. The Fimreite study establishes the lowest adverse concentration observed in avian eggs. Hoffman and Moore (1979) externally applied mercury to mallard eggs and found a dose related effects on survival, growth and abnormal development. The lowest dose applied which effected survival was 27 micrograms. Given an average mallard egg weight of 55 grams this dose corresponds to about 0.5 µg/g.

Reproductive effects may extend beyond the embryo to adversely effect the juvenile survival rates. Mercury in the eggs of mallards caused brain lesions in hatched ducklings. Mallards were fed 3.0 µg/g methylmercury dicyandiamide over two successive years. Mercury was accumulated in the eggs to an average of 7.18 and 5.46 µg/g on a wet weight basis in 2 successive years. Lesions included demyelination, neuron shrinkage, necrosis and hemorrhage in the meninges overlying the cerebellum (Heinz 1975). Bouton *et al*. (1999) reported significant behavioral effects on juvenile egrets in captive feeding studies at both high (5,000 µg/g) and low (500 µg/g) dose concentrations of mercury in the diet. Effects in the low dose group included lethargy, reduced motor skills, reduced packed cell volume, decreased appetite and changes in time spent standing vs. sitting. Low dose birds were also less likely to hunt and more likely to seek shade. An observation of significance in the Everglades appears to be that once feather growth ceases, mercury may pose a greater threat to fledgling birds as circulating levels of mercury in the blood are no longer sequestered in the growing feathers. This may be a critical stage for birds as they must learn to hunt and survive on their own at this time.

Mammals: Methylmercury toxicity in mammals is primarily manifested as central nervous system damage, sensory and motor deficits, and behavioral impairment (Wren et al, 1988; Wren et al., 1986). Animals initially become anorexic and lethargic. Muscle ataxia, motor control deficits, and visual impairment develop as toxicity progresses, with convulsions preceding death (O'Conner and Nielsen, 1981; Wobeser et al., 1976). Smaller carnivores are more sensitive to methylmercury toxicity than larger species, as reflected in the shorter time to onset of toxic signs and time to death. Dietary concentrations of 4,000 to 5,000 µg/g methylmercury were lethal to mink and ferrets within 26 to 58 days, whereas otters receiving the same concentration survived an

average of 117 days (Wren et al., 1988; Wren, 1986). Methylmercury is readily transferred across the placenta and concentrates selectively in the fetal brain. Mercury concentrations in the fetal brain were twice as high as in the maternal brain for rodents fed methylmercury (Yang et al., 1972). Reproductive effects of methylmercury in mammals range from developmental alterations in the fetus, which produce physical or behavioral deficits after birth, to fetal death (Eccles and Annau,1987; Chang and Annau, 1984).

The behavioral deficits produced by prenatal exposure to methylmercury are known mostly from work with rodents and monkeys. Rats and mice exposed via the diet or by gavage at various times during gestation period showed retarded righting reflex, impaired or retarded swimming ability, decrease in spontaneous activities, impaired maze and avoidance learning, and deficits in operant learning (Shimai and Satoh, 1985). The use of primates to study the behavioral teratology of methylmercury has permitted more extensive investigations. Infant crab-eating macaques (*Macaca nemestrina*) born to females exposed to 50 or 70 µg/g/day of methylmercury had blood methylmercury levels of 1,690 µg/L at birth and 1,040 µg/L at the time of testing. The exposed macaques had significant deficits of visual recognition memory compared to controls (Gunderson et al., 1988). Cynomolgus monkeys (*Macaca fascicularis*) born to females given 50 µg/kg/day methylmercury showed more non-social passive behavior and less social play than non-exposed monkeys (Burbacher et al., 1990). Adult macaques dosed with 0.24 to 1.0 µg/g methylmercury at twice-weekly intervals for up to 73 weeks first experienced constriction of visual field, as has been reported by methylmercury-intoxicated humans, an effect that was reversible if exposure was discontinued. At higher or more prolonged doses, visual field constriction became permanent, and visual thresholds were altered, reflecting damage to neurons in the visual cortex (Merigan et al., 1983).

Bioaccumulation of mercury

Both organic and inorganic mercury bioaccumulate, but methylmercury accumulates at greater rates than inorganic mercury. Most mercury in fish or wildlife organisms is in the form of methylmercury (Bloom, 1995) as this form is more efficiently absorbed (Scheuhammer, 1987) and preferentially retained (Weiner, 1995). Much of the inorganic mercury found in some organisms such as procellariiform birds (albatrosses, shearwaters, and petrels) may have actually been originally accumulated as methylmercury and then demethylated by the organism. The bacterial rates of production of methylmercury in water and sediment matrices ultimately determines the potential of an aquatic system to develop a mercury bioaccumulation problem. Food chain transfer is the most important exposure pathway in all ecosystems (EPA, 1997b). Methylmercury is one of the rare compounds which not only bioaccumulates but also biomagnifies across trophic levels such that field measured BAFs for methylmercury are commonly in the millions for top trophic level fish (Nichols et al., 1999).

Table 5. Median bioaccumulation factors for fish presented in the Mercury Study Report to Congress (EPA, 1997b).

Hg form	BAF trophic level 3 fish*	BAF trophic level 4 fish*
Total mercury	124,800	530,400
Methyl mercury	1,600,000	6,800,000

Aquatic ecosystems tend to have higher rates of bioaccumulation and biomagnification than do terrestrial ecosystems (EPA, 1997b). Explanations for this phenomenon include the fact that fish store most mercury as methylmercury in their muscle while mammals and birds store much of their methylmercury burden in feathers and fur, items poorly digested or rarely eaten. Aquatic systems have more complex food webs and more trophic levels, and the primary producers in aquatic systems may themselves accumulate more mercury from water and sediment than do soil based primary producers in terrestrial systems (EPA, 1997b). Top predators in aquatic systems therefore are at greatest risk from mercury bioaccumulation. Mercury concentrations in blood greater than 1,000 µg/L and in eggs greater than 0.5 µg/g are considered harmful. In liver 5 µg/g is considered a conservative threshold for potential adverse effects to waterbirds (Wolfe *et al.*, 1998).

Listed wildlife species which are high trophic level predators in aquatic systems of California include one mammal, six birds, and two reptiles. These are the southern sea otter, bald eagle, California least tern, California brown pelican, California clapper rail, light-footed clapper rail, Yuma clapper rail, giant garter snake, and San Francisco garter snake.

Bioaccumulation Hazards of Mercury to Fish: Diet is the primary route of methylmercury uptake by fish in natural waters, contributing more than 90 percent of the methylmercury accumulated. The assimilation efficiency for uptake of dietary methylmercury in fish is probably 65 to 80 percent or greater. To a lesser extent, fish may obtain mercury from water passed over the gills, and fish may also methylate inorganic mercury in the gut (Wiener and Spry, 1996). Developing embryos are the most vulnerable life stage to mercury exposure. In all vertebrates, including fish, the transfer of methylmercury to the embryo represents the greatest hazard. In addition to the hazard to top avian reptilian and mammalian predators in aquatic systems, fish and amphibian species, particularly long lived species, may be at risk from mercury bioaccumulation and biomagnification. Even in fish, "methylmercury derived from the adult female probably poses greater risk than waterborne mercury for embryos in natural waters" (Wiener, 1995). This is likely true for amphibians, including the federally listed California red-legged frog. For this reason alone mercury criteria needed to protect aquatic life must consider maternal bioaccumulation rates in adult fish. Sublethal and lethal effects on fish embryos are associated with mercury residues in eggs that are perhaps 1 percent to 10 percent of the residues associated with toxicity in adult fish (Weiner, 1995). Mercury intoxicated rainbow trout have between 4 and 30 µg/g in whole bodies, while intoxicated embryos contain 0.07 to 0.1 µg/g (Weiner, 1995). Listed fish species with long life spans are potentially at risk from mercury bioaccumulation. Listed fish species potentially at risk of mercury bioaccumulation at concentrations permissible under the CTR criteria include

listed salmonids, as well as the bonytail chub, razorback sucker, shortnose sucker, Lost River sucker and the Sacramento splittail.

While the Mercury Study Report to Congress (EPA, 1997b) generated data on a range of national bioaccumulation factors, that report emphasized the value of developing site specific and field derived bioaccumulation factors when developing criteria for specific regions. Factors which affect the site specific bioaccumulation factors within a given ecosystem are many and varied. Factors proposed to effect bioaccumulation rates include the number of trophic levels present and food web structure of the aquatic ecosystem, the abundance of sulfur reducing bacteria and the concentration of sulfates, dissolved oxygen, water temperature, organic carbon availability, pH, the nature of the mercury source and other parameters (Porcella *et al*., 1995).

In the absence of site-specific bioaccumulation factors for mercury, EPA recommended default BAFs using the bioaccumulation factors in (EPA 1997b) (see table 5). In order to develop a site-specific bioaccumulation factor, concomitant measurements of mercury in fish and water are needed. Water measurements need to employ ultra clean sampling techniques (Gill and Bruland, 1990) and picomolar quantification methods of mercury determination in water (Bloom, 1989). In this regard there is a clear need for EPA to promulgate a new analytical method for mercury under the CWA which will have appropriate detection limits in water and address the problems of sample contamination in the current method.

While EPA's current human health criterion per the draft CTR continue to use bioconcentration factors from older lab studies, the EPA used bioaccumulation factors to assess ecological and human health risk for the Mercury Study Report to Congress. That report recommended the use of field derived bioaccumulation factors. The Services are aware of currently available, scientifically defensible field data which may likely permit calculation of site-specific bioaccumulation factors for mercury at a number of California locations. These locations include Clear Lake, Lake Nacimiento, Cache Creek, Walker Creek, Marsh Creek, Lake New Almaden, the New Almaden Mine area, Marsh Creek, the Sacramento River, the Petaluma River, Central San Francisco Bay, South San Francisco Bay (Cal/EPA, 1997), Davis Creek Reservoir, Snake Creek, Lake San Antonio and Las Tablas Creek (Gill and Bruland, 1990) as well as the Yuba River, the Feather River, the American River, and the Cosumnes River (Slotten et al., various reports to Central Valley Regional Board 1999). Ongoing studies funded by CalFed may support the development of such bioaccumulation factors for the Sacramento/San Joaquin delta area within the next two years.

Bioaccumulation Hazards of Mercury to Reptiles and Amphibians: The maternal transfer of methylmercury is likely to occur in amphibians and reptiles as it does in fish and birds. The Service is not aware of any available data on adverse effect residue concentrations in amphibians or reptiles which would at this time permit a calculation of an effect threshold for the red-legged frog, giant garter snake or San Francisco garter snake. The USFWS has conducted a study with the Biological Resource Division of United States Geologic Survey (USGS) within the Cache Creek drainage on mercury bioaccumulation within the watershed. Results from this study show

maximum whole body mercury concentrations in foothill yellow-legged frogs (*Rana boylii*) of 0.79 µg/g ww and 1.29 µg/g in bull frogs *(Rana catesbeiana*). In the absence of specific amphibian data the Services would recommend applying a fish model to assessing the risk to amphibian eggs laid in water and an avian risk model to evaluate impacts to predatory snakes in aquatic environments.

Bioaccumulation Hazards of Mercury to Birds: Mercury is transferred to avian eggs in proportion to the maternal dose (Walsh, 1990). Almost all of this mercury is methylmercury (Schwarzbach et al., 1997; Wolfe et al., 1998). While some of this egg mercury represents maternal body burden, much of it reflects maternal diet during the immediate pre-laying period. Trophic position, and mercury sources of contamination on the breeding grounds are the most significant factors in predicting mercury concentrations in bird eggs. Only relatively minute mercury concentrations are required to impair eggs.

There is substantial data on mercury in avian eggs of a number of species throughout California. A few of these are federally listed species. These data are summarized in the mercury appendix of this document. These data show that exclusively piscivorous birds typically face the greatest risk, followed by partially piscivorous birds. Clapper rails, a benthic omnivore and partially piscivorous bird, can also achieve very high levels of egg mercury where sediment methylmercury production is high. The California clapper rail in south San Francisco Bay has the maximum single egg concentration of mercury measured in any California egg at 2.5 µg/g (fww) (Schwarzbach et al., 1997). Other listed species for which egg mercury data exist in California include the light-footed clapper rail, the Yuma clapper rail, and the least tern. Data for eleven different bird species (Schwarzbach et al., 1997) overwhelmingly show that birds nesting in San Francisco Bay, including the least tern and the California clapper rail, are at much greater risk of mercury bioaccumulation than their cohorts nesting elsewhere in California. Data also indicate that Elkhorn Slough is nearly equally mercury impaired with regard to excessive bioaccumulation of mercury in fish eating birds (Caspian terns). The effects of the CTR mercury criteria, as proposed, will leave this condition unchanged.

We are unaware, at this writing, of bald eagle egg data for California. The only mercury data available to the Services is blood mercury data from the Klamath Basin (Frenzel and Anthony, 1989). These data showed a mean concentration of 2,290 µg/L. This is a concentration 7.5 times higher than bald eagles kept in captivity (Frenzel and Anthony, 1989) and well over the concentration of 1,000 µg/L suggested as harmful.

Bald eagles in California are likely to be the species with the greatest concentrations of mercury in eggs as nesting pairs occur at mercury contaminated reservoirs throughout the Coast Range and eagles occupy the highest trophic position in those systems. The proposed CTR mercury criteria will leave this condition unchanged, and likely not protect eagles from bioaccumulation. This conclusion is supported by the Mercury Study Report to Congress (EPA, 1997b) which developed an estimated total (as dissolved) mercury water concentration of 1.05 ng/L to protect the bald eagle from the bioaccumulation of mercury throughout its range. While site-specific factors may

vary, it is unlikely that site specific bioaccumulation factors would lead to a new criterion above EPA's 50 ng/L human health criterion proposal.

Reproduction is the most sensitive endpoint and mercury accumulated in egg is the best predictor of mercury risk to embryo survival. Egg mercury measurements are superior to measurements of mercury concentration in potential prey items as proportions of possible prey in diet are not always known. One of the significant factors enhancing risk of mercury to the avian embryos is the lack of any protective detoxification mechanism in the avian egg once mercury is deposited there. The lowest adverse effect concentration in avian eggs is 0.5 µg/g (fww) (Fimreite, 1971). The no adverse effect concentration in avian eggs is unknown. Mean fresh wet weight mercury concentration in failed eggs of the California least tern in San Francisco Bay in 1994 was 0.74 µg/g (fww). California clapper rail failed eggs in 1992 had a mean of 0.63 µg/g mercury in eggs.

A mercury bioaccumulation factor (BAF) for water or sediment to egg may be derived on a site- and species-specific basis. The USFWS has derived a mercury BAF for water to least tern eggs in San Francisco Bay (described below). A sediment BAF of 1,435 (on a ww basis) for methylmercury accumulation in California clapper rail eggs from sediment has been previously described elsewhere (Schwarzbach et al., 1996). These BAFs can be used in equations together with an estimated no observable adverse effect level (NOAEL) for mercury in avian eggs to estimate a safe concentration in water or sediment for the respective species. Alternatively, one may use the equations described and used in the Mercury Study Report to Congress (USEPA, 1997b) to derive an estimate of a safe concentration for mercury in water. These equations rely on dietary concentrations and bioaccumulation factors to fish together with a safe dietary daily dose estimate. These two methods are compared below to derive a water criterion for mercury protective of the least tern in San Francisco Bay. All of these methods suggest that for the mercury criterion to be protective of wildlife the concentrations would need to be substantially lower than proposed in the CTR.

Bioaccumulation Hazards of Mercury to Mammals: Mammals that forage within aquatic ecosystems are at greatest risk of mercury bioaccumulation. In mammalian tissues the greatest concentrations of mercury are usually found in liver and kidney. Mammals that consume fish, or mammals that consume mammals that consume fish are generally at greatest risk.

O'Conner and Nielsen (1981) found that length of exposure was a better predictor of tissue residue level than dose in otters but higher doses produced an earlier onset of clinical signs. A dose of 0.09 µg/g body weight (2 µg/g in diet as methylmercury) for 181 days was enough to produce anorexia and ataxia in two of three otters in a feeding study of river otters (*Lutra candensis*). Associated liver residues were 32.6 µg/g (O'Conner and Nielsen, 1981). Concentrations of 21 to 23 µg/g in kidney and liver were associated with liver and kidney histologic alterations in Rhesus monkeys (Rice *et al*., 1989). Muscle ataxia, motor control deficits, and visual impairment develop as toxicity progresses with convulsions preceding death. River otters fed 8 µg/g methylmercury died within a mean time of 54 days. Associated liver concentrations were 32.3 µg/g (O'Conner and Nielsen, 1981). While 8 µg/g or even 2 µg/g seems

a higher concentration than what southern sea otters are likely to encounter in their prey, the duration of sea otter exposure in the wild is life-long. As indicated by mercury residues in sea otter livers, and laboratory feeding studies showing the importance of duration of dose, life long multi-generation exposures to elevated mercury in diet may produce elevated mercury in tissues and the attendant adverse effects. A long term exposure to mercury in the diet may result in the most exposed individuals experiencing decreased motor coordination, reduced sensory and mental acuity, impaired kidney function, ataxia, anorexia and even death.

In California the listed mammal which may be at greatest risk from mercury is the southern sea otter. The California sea otter population is endangered and population levels are declining. Sea otters forage in the nearshore marine environment, from the intertidal to depths exceeding 60 feet. At Elkhorn Slough, otters are often found foraging well within the slough. While sea otters, unlike river otters, are not exclusively piscivorous, they are opportunistic foragers on mussels, snails, clams, crabs, squids, sea urchins, star fishes and slow moving fish among other organisms (Estes, 1980; Zeiner, 1990). In captivity sea otters consume 15 to 35 percent of their body weight in food daily (Lensink 1962). The metabolic demands of sea otter existence may thus result in elevated risk of sea otter contaminant loading, although a lower fraction of the mercury consumed by omnivores is likely to be methylmercury. Wren (1986) suggested normal mercury concentrations in river otter livers were 4 µg/g (fww) or below. Livers collected from sea otters found dead along the central California coast had a maximum mercury concentration of (60 µg/g) (Mark Stephenson pers comm 1998). Of 125 sea otter livers examined for mercury on the California coast, 56 had concentrations greater than 4 µg/g and 30 had concentrations over 10 µg/g. Four had concentrations over 30 µg/g.

Estimates of Mercury Criteria Protective of listed Fish and Wildlife Species:

The proposed CTR as published in the federal register states: "This rule is important for several . . . reasons. Control . . . is necessary to achieve the CWA's goals and objectives. Many of California's . . . waters have elevated levels of toxic pollutants. Recent studies . . . indicate that elevated levels of toxic pollutants exist in fish tissue which result in fishing advisories or bans." Many of these advisories exist due to mercury bioaccumulation which is elevated in a number of water bodies in California. San Francisco Bay trophic level 3 fish average 0.140 µg/g (San Francisco Regional Water Quality Control Board, 1995), a level 2.7 times the national average and 1.8 times the concentration of methylmercury in trophic level 3 fish of 0.077 µgHg/g, (Nichols et al., 1999) associated with EPA's wildlife value in water. It is the Services' opinion that the effect of the proposed action (CTR) would be to effectively leave this condition (fish advisories and elevated mercury in trophic level 3 fish) unlikely to change. Further it is the opinion of the Services that sufficient data is available to allow preliminary calculation of protective criteria in California which take into account site-specific bioaccumulation to fish.

Below we calculate a bioaccumulation based mercury criterion to protect salmonids and a bioaccumulation based criterion to protect the California least tern in San Francisco Bay. While additional research would no doubt improve the confidence in the calculations below, it is readily

apparent from both the Mercury Study Report to Congress, and our calculations with available data, that the proposed criteria in the draft CTR would be too high to protect many top predators in aquatic systems, including some listed species.

Estimating a Bioaccumulation Based Effect Concentration in Salmonids:

Neither the aquatic life criteria nor the human health criterion for mercury address the hazard of bioaccumulation of mercury to fish themselves, but only to the human consumers of fish. Where fish effects are considered in the aquatic life criterion it is only through direct waterborne toxicity. Mercury residue concentrations have been observed in mercury intoxicated trout of 4 µg/g (Wiener, 1995). Brook trout with whole body concentrations of 2.7 µg/g exhibited reproductive impairment (McKim et al., 1976). Using the default BAF_4 from USEPA (1997b) we derive below a water concentration of 5 ng/L total dissolved mercury which could be associated with reproductive impairment.

$$\text{Adverse effect concentration [T-Hg] in water for trophic level 4 fish} = \frac{\text{Toxic to fish Hg whole body conc.}}{BAF_4}$$

$$= \frac{2{,}700 \text{ ng/g}}{530{,}400 \text{ ng/g/µg/L}}$$

$$= 0.005 \text{ µg/L}$$

$$= 5 \text{ ng/L}$$

An examination of the data from rivers tributary to San Francisco Bay in 1996 (SFEI, 1997b) indicates that the dissolved component of total mercury varies seasonally but averages 19 percent 13 percent and 7.5 percent for the Sacramento, Napa, and Petaluma Rivers respectively. Using these mean ratios, corresponding total mercury effect concentrations in unfiltered water of these northern California rivers would be estimated at 26, 38, and 66 ng/L. Appropriately protective criteria should be below the effect concentrations. EPA's 51 ng/L criterion for human health would be below only the Petaluma River effect estimate. Dividing the effect concentrations by a safety factor of 2 would result in a fish protective criterion lower than the CTR human criterion (51 ng/L) in all three rivers.

Estimating a Bioaccumulation Based Mercury Criterion for Wildlife Species: Comparison of Two Estimates Using an Oral Dose Model and an Egg BAF Model in the California Least Tern in San Francisco Bay.

A wildlife criterion is defined by EPA to be the highest concentration of a substance that causes no significant reduction in growth, reproduction, viability or usefulness of a population of animals exposed over multiple generations. For a species listed as endangered the failure to achieve

concentrations at or below an appropriate wildlife criterion may be critical to future survival of the species. While the final Mercury Study Report to Congress (USEPA, 1997b) developed a wildlife criterion for the bald eagle, the Services offer the following calculations using California specific data for the least tern and San Francisco Bay to illustrate that EPA's Great Lakes wildlife criteria are more nearly appropriate than the human health criterion suggested by EPA as protection for California's listed wildlife species.

For the purposes of example in this opinion, the Services have taken mercury data in water and trophic level 3 fish (shiner surf perch, a prey item of the California least tern) from the San Francisco Bay Regional Monitoring Program. Water mercury data collected by the San Francisco Estuary Institute (SFEI) in the spring of 1994 from 6 locations within central San Francisco Bay were also used. Fish mercury concentrations in shiner surf perch were matched with the two or three closest water sampling locations due to the fact that fish are mobile and water concentrations vary. Springtime water values were used because this is when California least terns are nesting in the bay (April BAFs also appear to generally be intermediate between February and August values in the Central Bay). Dry weight and wet weight bioaccumulation factors for mercury in shiner surf perch were calculated from the Regional Monitoring Program's data and are presented in Table 6.

Table 6. Dry weight and wet weight bioaccumulation factors for trophic level 3 (BAF_3)[@] fish in Central San Francisco Bay.

Fish Collection Location	Representative Water Collection Points	BAF_3 (DW) Total unfiltered Hg	BAF_3 (WW) Total unfiltered Hg
Richmond Harbor	Point Isabel, Red Rock , Yerba Buena	137,311	30,483
Berkeley Pier	Point Isabel, Red Rock, Yerba Buena	118,098	27,163
Oakland Inner Harbor	Yerba Buena, Alameda	181,840	42,551
Oakland Middle Harbor	Yerba Buena, Alameda	72,290	20,530
Double Rock	Alameda, Oyster Point	76,319	18,088
Islais Creek	Yerba Buena, Alameda, Oyster Point	53,917	13,425
Geometric Mean for central SF Bay		97,723 [!]	23,659

@ Trophic level 3 fish are non-piscivorous foraging fish.

+ Mercury Data from 1994 Regional Monitoring Program (RMP) in SF Bay winter and spring of 1994 (SFEI, 1997).

! Geometric mean dry weight factor is used in least tern criterion equation because the diet estimate for terns was based upon allometric equations using dry weight.

The following equation is used to calculate a wildlife criterion for least terns. This equation is identical to the one described in the Mercury Study Report to Congress, Volume VI (USEPA 1997b).

$$WC = \frac{TD \times (1/UF) \times Wt_A}{W_A + [(FD_3)(F_A \times BAF_3) + (FD_4)(F_A \times BAF_4)]}$$

WC = Wildlife criterion (units as calculated will be in mg/L; convert to µg/L)

Wt_A = Average species weight (kg)

W_A = average daily volume of water consumed (L/d)

F_A = average daily amount of food consumed (kg/d) (dry weight)

FD_3 = fraction of the diet derived from trophic level 3

FD_4 = fraction of the diet derived from trophic level 4

BAF_3 = aquatic life bioaccumulation factor for trophic level 3 (dry weight)

BAF_4 = aquatic life bioaccumulation factor for trophic level 4

TD = Threshold dose (mg/kg Body Wt/day). Ideally the threshold dose should be a bounded NOAEC (No observed adverse effect concentration). If however a NOAEC is not known then an uncertainty factor may be appropriately applied to a LOAEC (Lowest observed adverse effect concentration).

UF = Uncertainty Factor

The EPA procedure provides that in the absence of a NOAEC a LOAEC may be used with the addition of an uncertainty factor. Other uncertainty factors may be applied where there is interspecies uncertainty and when extrapolating from subchronic to chronic exposures.

Equation Values used for Least Tern

California least terns, a federally listed species, are the smallest members of the subfamily Sterninae (family Laridae), measuring about 22.9 cm (nine inches) long with a 50.8 cm (20 inch) wingspread and body weights ranging between 45 and 55 g. They are exclusively piscivorous and typically consume such trophic level 3 fish as topsmelt, anchovy, surf perch and jacksmelt.

Trophic level 3 fish are those which consume aquatic invertebrates, and planktivores. Thus, for the least tern in this analysis:

FD_4 = 0 and **FD_3 = 1.0**.

Using an average body weight of 0.05 Kg the F_a value for food consumption per day (dry weight) may be calculated using allometric equations for seabirds found in Nagy (1987) :

$g/d = 0.495(Bw)^{0.704}$. This results in **F_a = 0.0078 kg/day.**

Allometric equations are also used to generate an estimate of W_A . The following equation is from Calder and Braun, 1983:

$L/day = 0.059(BW)^{0.67}$. This results in **W_A = 0.007 L/day**.

A field derived BAF from central SF Bay for total mercury (for comparative purposes) was derived from synoptic sampling of fish (shiner surf perch) and water using ultra clean techniques at 6 central bay locations by the Regional Monitoring program in 1994 (Table 6). This BAF was derived from the geometric mean of these 6 sites. While field BAFs vary somewhat, USEPA (1997b) recommends using the geometric mean BAF where exposure concern is for repeated ingestion. The dry weight geometric mean BAF for total unfiltered mercury to shiner surf perch in Central SF Bay is 97,723 (Table 6). The allometric equations estimating food consumption of the tern are dry weight based, thus dry weight mercury concentrations were used to derive the dry weight BAF.

BAF_3 (dw) = 97,723 as total Hg (field derived, Central SF Bay).

(Note: A total mercury criterion is developed here to allow comparison of a sample wildlife criterion with the human health criterion proposed by EPA. Future development of wildlife criteria for California should probably be based upon a dissolved mercury or dissolved methylmercury concentration in water.)

The threshold dose value is from a three generation study feeding study in mallards with methylmercury dicyandiamide (Heinz, 1979). The lowest dose resulted in adverse effects on reproduction and behavior, therefore, this concentration represents a LOAEC not a NOAEC. This is the value used by EPA to calculate wildlife criteria in the final Mercury Study Report to Congress (USEPA, 1997b).

TD = 0.078 mg/kg/day

UF = 3 The EPA procedure provides that in the absence of a NOAEC a LOAEC may be used with the addition of an uncertainty factor. Other uncertainty factors may be applied where there is interspecies uncertainty and when extrapolating from subchronic to chronic exposures. Because the field species in this case, the least tern, is a piscivorous bird and fish eating birds may have

greater capacity to demethylate mercury, no interspecies uncertainty factor is applied. Because the tested threshold dose was derived from a chronic 3 generation exposure no uncertainty factor for exposure duration is applied. An uncertainty factor of 3 is applied because the TD is a LOAEC not a NOAEC. The detailed reasoning behind the uncertainty factor of 3 is provided in USEPA (1997b) and Nichols et al. (1999).

Completing the equation yields:

$$WC = \frac{0.078 \text{mg/kg/day} \times [1/3] \times 0.05 \text{kg}}{0.007 \text{ L/d} + [1.0(0.0078 \times 97{,}723)]} = 0.000001705 \text{ mg/L as dissolved total Hg}$$

WC = 0.00171 µg/L or **1.71 ng/L** total unfiltered Hg

Without using the uncertainty factor of three, the equation produces an effect threshold concentration for mercury in water where "take" may be estimated to occur for the least tern. This concentration is 5.11 ng/L as a geometric mean.

We conclude that using an oral dose model per the methods of USEPA, 1997b, a wildlife criterion that might be protective of California least terns would be 1.71 ng/L total unfiltered mercury.

Tern egg bioaccumulation method: An alternative method to calculate a wildlife criterion is to use the egg residues from the field and divide by the associated water mercury concentrations to develop an egg/water bioaccumulation factor. The egg/water BAF can then be used with established values of egg residues associated with embryo toxicity to determine a wildlife criterion. This method can then be assessed and compared with the dietary method of EPA for independent validation.

Six fail-to-hatch California least tern eggs from the nesting colony at Alameda Naval Air Station in 1994 were analyzed for mercury content. The wet weight mean concentration was 740 ng/g and concentrations ranged from 390 ng/g to 1,300 ng/g (Schwarzbach et al., 1997). Water mercury data in 1994 was collected as part of the Regional Monitoring Program by the San Francisco Estuary Institute (SFEI) at a number of stations in San Francisco Bay. The mean mercury concentration in unfiltered water in April among the following five central bay sites (Point Isabel, Red Rock, Yerba Buena, Alameda and Oyster Point) was 4.7 ng/L. This value is used to estimate the water mercury concentration for the BAF calculation. The April data was selected because of their proximity to the egg laying season for terns.

The following equations are used to calculate a protective criterion for total mercury in water. Wet weight values are used because toxic thresholds for mercury in eggs are typically expressed in wet weight.

$$\text{species-specific field BAF} = \frac{\text{measured egg concentrations}}{}$$

for Ca. least terns measured water concentration

$$= \frac{740 \text{ ng/g}}{4.7 \text{ ng/L}} = 157 \text{ ng/g/ng/L}$$

A water criterion can now be derived by dividing the avian egg NOAEL by the field BAF. Unfortunately there is no known bounded avian egg NOAEL. The LOAEL however is 500 ng/g (ww). Using a LOAEL/NOAEL ratio for mercury concentrations in avian egg of two, one obtains a calculated NOAEL of 250 ng/g.

$$\frac{\text{NOAEL concentration in egg}}{\text{Field egg/water BAF}} = \frac{250 \text{ ng/g}}{157 \text{ ng/g/ng/L}} = 1.59 \text{ ng/L total mercury}$$

Dividing the NOAEL by the BAF results in a calculated water criterion concentration of 1.59 ng/L total mercury, a value comparable to the 1.71 ng/L result of the oral dose model presented above.
Without the uncertainty factor of 2, an effect threshold of 3.2 ng/L is calculated as total mercury (in unfiltered water).

EPA has calculated a piscivorous wildlife criterion value of 0.05 ng/L as methylmercury or 0.641 ng/L total "aqueous" (dissolved) mercury for protection of piscivorous wildlife (USEPA, 1997b). The wildlife criterion calculated by EPA in the Mercury Study Report to Congress was not released as a final report prior to the publication of the draft CTR in the federal register (USEPA, 1997c) and the mercury criterion for California water bodies as proposed in the CTR does not reflect this now available science. This "criterion value" has thus far been officially issued only in a report to Congress, not as guidance to the states as a basis for regulating water quality.

The criteria calculations presented above were done to evaluate the degree of protectiveness of EPA's CTR mercury criteria for a listed piscivorous species using site-specific bioaccumulation factors; to compare that site-specific criterion with criteria developed in the Mercury Study Report to Congress; and to evaluate the comparative usefulness of the egg bioaccumulation model with the oral dose model used by EPA in predicting mercury toxicity to avian reproduction. If comparable, this method may serve as a valuable alternative to the oral dose model for avian species where egg mercury and water data are available but dietary concentrations are not known. This model is most useful in predicting toxicity of bioaccumulated compounds to birds when the most sensitive endpoint is embryo toxicity.

The California least tern is exclusively piscivorous, or nearly so, and therefore tern mercury bioaccumulation, unlike clapper rail, is most directly dependent upon mercury concentrations in the water column. Another advantage of using the tern as a model species for estimating a water based criterion is that mercury data in fish, water and eggs exist from the same time period which allow a calculation of mercury criteria using both models. The three sub-species of clapper rails

(Yuma, light-footed, and California subspecies) have a mercury exposure pattern complicated by their benthic foraging habits and minor piscivory. For the bald eagle EPA has already developed a criterion (USEPA, 1997b). The California least tern diet overlaps in significant ways the potential diet and mercury exposure levels of the federally protected marbled murrelet.

The wildlife criteria calculated in the Mercury Study Report to Congress (1997b) was based on risk estimates to six species, two species of fish eating mammals (mink and river otter) and four species of fish eating birds (loons, bald eagles, kingfisher and osprey). Criteria were calculated on a methylmercury basis using an oral dose model similar to that used in the Great Lakes Initiative (USEPA, 1995b). Table 7 compares results of the two models with the various wildlife criteria developed by the USEPA (1997b) and the mercury criteria for California water bodies as proposed in the CTR.

Calculated water concentrations protective of terns from each of the two methods produce similar numbers for total mercury. The calculated wildlife criterion using EPA's oral dose model is 1.71 ng/L (oral dose model) while the egg bioaccumulation model estimates 1.59 ng/L (BAF model). These numbers are also in close agreement with EPA's overall number of 2.3 ng/L for piscivorous mammals and birds and clearly indicate that mercury criteria as proposed in the CTR are between one and three orders of magnitude under protective for listed wildlife species including the least tern and bald eagle. The Services conclude that the egg BAF model is capable of calculating a criterion comparable to the oral dose model prediction. The Services further conclude that criteria developed in the Mercury Study Report to Congress (1997b) would likely be sufficiently protective for the least tern and other piscivorous wildlife species in California.

Table 7. Mercury criteria concentrations in fresh water.

Source	"protected entity"	dis. methyl Hg	dis. total Hg	unfiltered total Hg	basis of criteria
USEPA,1997b.	loon	0.067 ng/L	0.859 ng/L^	3.09 ng/L*	Oral dose model
"	eagle	0.082 ng/L	1.051 ng/L^	3.78 ng/L*	"
"	kingfisher	0.027 ng/L	0.346 ng/L^	1.24 ng/L*	"
"	osprey	0.067 ng/L	0.859 ng/L^	3.09 ng/L*	"
"	mink	0.057 ng/L	0.73 ng/L^	2.63 ng/L*	"
"	river otter	0.042 ng/L	0.54 ng/L^	1.94 ng/L*	"
"	Piscivorous Wildlife	0.05 ng/L	0.641 ng/L^	2.3 ng/L*	"
FWS (oral dose)	Ca. least tern		0.46 ng/L*	1.71 ng/L	oral dose model

FWS (egg BMF)	Ca. least tern		0.44 ng/L*	1.59 ng/L	egg BAF model
CTR	aquatic life (chronic)		770 ng/L	2,772 ng/L*	waterborne toxicity
CTR	aquatic life (acute)			5,040 ng/L*	waterborne toxicity
CTR	human health			50 ng/L	1980 BCFs
Former CA Standards	Aquatic Life (chronic)			12 ng/L	literature evaluation
Former CA Standards	Aquatic Life (acute)			2,400 ng/L	literature evaluation

^ EPA methylmercury values are converted to dissolved total mercury by using 0.078 as an estimate of the fraction of methylmercury as a proportion of total mercury. This was EPA's "best" estimate (USEPA, 1997b). Methylmercury data for waters in San Francisco Bay is not available.

*Dissolved total mercury is converted to total unfiltered mercury and vice versa for all values by multiplying or dividing as appropriate by the ratio of total to dissolved (3.6) mercury to be consistent with conversion factor used in developing tern criteria. Values from 1994 RMP data from central San Francisco Bay (SFEI, 1997a).

Summary of Mercury Effects to Listed Species

Birds

Bald Eagle: The bald eagle is a generalized predator/scavenger primarily adapted to edges of aquatic habitats. Its primary foods, in descending order of importance, are fish (taken both alive and as carrion), waterfowl, mammalian carrion, and small birds and mammals.

The Klamath Basin in northern California and southern Oregon supports the largest wintering population of eagles in the lower 48 states, where up to 1000 birds may congregate at one time. Elevated mean mercury concentrations of 2.25 µg/L in the blood of bald eagles has been documented in the Klamath Basin (Frenzel and Anthony, 1989). Bald eagle exposure to elevated concentrations of mercury in California is likely, particularly in eagles wintering and breeding at coastal mountain reservoirs and associated watersheds. This exposure however, is poorly documented in eagle tissue and egg residues of mercury.

Scattered smaller groups of wintering eagles occur near reservoirs, and in close proximity to large concentrations of overwintering migratory waterfowl. In recent years San Antonio Reservoir has become an important wintering area for bald eagles. An estimate of 50+ eagles regularly winter there. These eagles may be exposed to hazardous mercury concentrations in the diet by foraging at nearby Lake Nacimiento. Important breeding sites for bald eagles include Lake Nacimiento. Lake Nacimiento is mercury impaired, and has a human health fish consumption advisory due to

mercury: women are cautioned against consuming any large mouth bass and no one should eat more than 24 ounces of large mouth bass per month from this lake (Cal EPA public health warnings). USEPA (1997b) has developed a mercury criterion for water protective of bald eagles of 1.05 ng/L (as dissolved total mercury) but this recommendation was published after the CTR. The Service concludes EPA's proposed aquatic life and human health mercury criteria of 770 ng/L and 50 ng/L, respectively, in the CTR are not protective of bald eagles.

California Least Tern: California least terns are an exclusively piscivorous bird. Information presented above demonstrates that permissible concentrations of mercury in water under the CTR would produce elevated concentrations in tern eggs and prey sufficient to impair least tern reproduction. In the case of terns nesting in San Francisco Bay, mercury has already been measured in eggs with concentrations high enough to impair avian reproduction (> 0.5 µg/g). Concentrations in fail to hatch tern eggs from Alameda Naval Air Station in 1994 ranged from 0.4 to 1.24 µg/g fww with a mean of 0.74 µg/g. The current mercury threat is lower to least terns nesting in southern California. Eggs in 1994 from San Diego had mercury concentrations ranging from 0.12 to 0.26 µg/g with a mean concentration of 0.19 µg/g ww. However, permissible concentrations under the CTR could allow mercury concentrations in Southern California bays and estuaries sufficient to adversely effect tern reproduction. The Service has calculated a criterion value for the least tern of 1.71 ng/L using EPA methodology (EPA 1997b) and site specific bioaccumulation factors from central San Francisco Bay. Alternatively the Service has used tern egg data to calculate a criterion of 1.59 ng/L using an egg bioaccumulation model. These two criteria calculations developed independently confirm that EPA's criterion of 50 ng/L will not protect the least tern. The Service further concludes the mercury status of terns in San Francisco Bay would not be improved by the CTR.

California Clapper Rail: The extant range of the California clapper rail is restricted to marshes of the San Francisco Bay Estuary. California clapper rails feed almost exclusively on benthic invertebrates, are non-migratory and vulnerable to local particulate and waterborne mercury inputs. Mercury contamination in rails summarized above and in the mercury appendix of this document indicates California clapper rails have the highest concentration of mercury measured in a single egg of any species nesting within San Francisco Bay (Schwarzbach et al, 1997). Mean concentrations in 36 fail to hatch eggs in 1992 was 0.63 µg/g (fww). The percentage of non-viable eggs among south bay marshes in 1992 ranged from 24 to 38 percent. Based upon current mercury impairment, and the range of wildlife criteria values for mercury between 1 and 3 ng/L total mercury summarized above, the Service concludes that neither the proposed dissolved numeric aquatic criterion of 770 ng/L nor the total mercury criterion of 50 ng/L for human health, would improve the current mercury status of the rail. The Service further concludes the promulgation and adoption of these criteria for San Francisco Bay could reduce incentives for mercury emission control strategies that would benefit the rail.

Yuma Clapper Rail: With a biological profile very similar to the California clapper rail, the Yuma clapper rail is similarly vulnerable to mercury contamination of prey and eggs. There is reason to suspect potential for mercury contamination of Yuma Rail habitat in tributaries of the Colorado

River downstream of discharges into Bat Cave Wash. Additionally the elevated selenium concentrations, the interactive potential for selenium and mercury toxicity to avian embryos and the lack of protection afforded by the human health criterion for mercury to Yuma clapper rails leads the Service to conclude protective mercury criteria are needed for the Yuma clapper rail.

Light-footed Clapper Rail: With a biological profile very similar to the California clapper rail, the light-footed clapper rail is similarly vulnerable to mercury contamination of prey and eggs. While the Service knows of no current mercury threat to the light-footed clapper rail, the potential for future mercury concentrations to increase with adoption of the CTR leads the Service to conclude more protective criteria are needed for the light-footed rail. The non-migratory, benthic foraging niche and fragmented habitat of light footed rails places them at great risk of locally elevated concentrations of mercury within tidal marshes.

Marbled Murrelet: During the breeding season marbled murrelets forage in near shore environments including bays and estuaries on small fish and euphasid shrimp. They have also been known to forage to a minor degree on salmonid fry in freshwater environments. As a piscivorous bird, much of the discussion provided above for the least tern regarding the inadequacy of the CTR-proposed mercury criteria may also apply to the marbled murrelet.

Adverse impacts from increased permissible concentrations of contaminants as proposed in the CTR to prey species such as the Pacific sardine, herring, topsmelt, and northern anchovies, has the potential to significantly reduce long-term reproductive success of marbled murrelets (USDI-FWS, 1997). Adverse effects to prey species spawning and nursery habitats have the potential to impair population size and reduce recruitment throughout their range in California. The vulnerability of marbled murrelet populations in conservation zones 5 and 6, coupled with elevated concentrations of contaminants in spawning and nursery areas for murrelet prey species increase the risk of bioaccumulation of mercury and selenium. The synergistic effects of these contaminants pose a significant threat to marbled murrelet reproduction throughout conservation zones 5 and 6 and to a lesser degree in conservation zone 4.

Consequently, until species-specific data are collected or species-specific modeling is conducted for the marbled murrelet, a mercury criterion similar to that developed in this opinion for the California least tern or the Mercury Study Report to Congress must be viewed as the applicable guidance for protection of marbled murrelets.

Amphibians and Reptiles

Reptiles and amphibians remain the least studied vertebrates for mercury toxicity. It is also likely that aquatic food chain contamination by mercury would be the most significant pathway of exposure as would maternal transfer of methylmercury to the eggs. The Service believes a fish risk model may be most appropriate for assessing mercury hazard to amphibians such as the red-legged frog. This assessment may however be overly simplistic. Development of amphibians is unique among vertebrates in the occurrence of hormonally mediated ontogenetic metamorphosis

within the water column (Duellman and Trueb, 1986). Chronic studies in frogs of the effects of mercury contamination are generally lacking.

California red-legged frogs spend most of their lives in and near sheltered backwaters of ponds, marshes, springs, streams, and reservoirs. These types of environments are particularly vulnerable to mercury contamination due to favorable conditions for the conversion of inorganic mercury to methylmercury. Red-legged frogs are reduced to about 30 percent of their historical range with most of the remaining population limited to coastal drainages. Several hundred abandoned mercury mines of varying sizes and states of remediation or disrepair currently contaminate this region with both inorganic and methylmercury. These mines and associated contaminated landscapes present potential exposure pathways for mercury to the habitat of the red-legged frog. Mercury residue data in yellow-legged frogs downstream from abandoned mines in the Cache creek data cited above and provided in the mercury appendix indicate ranid frogs may bioaccumulate mercury in the vicinity of these mines. The Service therefore concludes appropriate mercury criteria are needed for protection of red-legged frogs.

The Service was not able to locate any published acute or chronic studies of mercury in snakes. Studies of mercury in garter snakes are needed to better evaluate the protection afforded to these species of proposed mercury criteria.

Fish

Based on the information presented above on the toxicity of mercury to salmonid fish at 100 ng/L concentrations, it would appear the aquatic life criterion is unprotective of listed salmonids and possibly other fish species as well (Weiner and Spry 1995). Based on the review of mercury bioaccumulation factors in fish, it appears that harmful degrees of maternal transfer of mercury to fish eggs and young could occur at concentrations below the lowest CTR criteria number for mercury (50 ng/L). Mercury intoxicated rainbow trout have between 4 and 30 µg/g in whole bodies, while intoxicated embryos contain 0.07 to 0.1 µg/g (Weiner 1995). Application of EPA bioaccumulation factors predicts reproductive adverse effect concentrations at 5 ng/L total aqueous mercury. Due to the potential for elevated concentrations of mercury in water and/or biota in a number of California water bodies, and due to the life history characteristics, the Services believe an exposure pathway exists for the following listed or proposed fish species: all runs and ESUs of coho and chinook salmon and steelhead trout, Little Kern Golden trout, Paiute cutthroat trout, Lahontan cutthroat trout, bonytail chub, unarmored threespine stickleback, shortnose sucker, Lost River sucker and the Sacramento splittail.

Mammals

Southern Sea Otter: Southern sea otters are known to forage at the mouths of freshwater systems as well as in shallow marine waters adjacent to the coast. California has abundant geologic sources of mercury and a long history of mercury contamination associated with mercury mining, particularly in the Coast Range. These sources of mercury often are coincidental with headwaters

of streams discharging to the central California coast. Livers collected from sea otters found dead along the central California coast range as high as 60 µg/g (Mark Stephenson, CDFG, pers comm 1998). Of 125 California coast sea otters examined for mercury in liver, 45 percent had concentrations greater than what may be considered a normal river otter ambient concentration of 4 µg/g. One fourth of these salvaged individuals had concentrations over 10 µg/g and 3 percent had concentrations over the 30 µg/g hepatic concentration associated with lethality. Acute mercury poisoning in mammals is primarily manifested in central nervous system damage, sensory and motor deficits, and behavioral impairment. Animals initially become anorexic and lethargic.

Sea otters are voracious consumers eating as much as 35 percent of their body weight per day. This high forage rate leaves them potentially vulnerable to dietary contaminant loading. The diet of sea otters consists of slow moving fish and invertebrates (Estes, 1980). Sea otters obtain about 23 percent of their water needs from sea water, making them vulnerable to impaired kidney function from inorganic mercury and cadmium. The proximity of otter foraging to elevated coast range discharges of mercury and cadmium places the otter at risk of dietary mercury and cadmium exposure. Given the potential for exposure and the documentation of elevated concentrations in a significant fraction of dead otters the Service concludes a mercury wildlife criterion comparable to that developed for piscivorous wildlife in the Mercury Study Report to Congress is needed for sea otter protection.

EPA modifications addressing the Services' April 9, 1999 draft Reasonable and Prudent Alternatives for mercury:

The above effect analysis evaluates the draft CTR as originally proposed in August of 1997. EPA has agreed by letter dated December 16, 1999, to modify its action for mercury per the following to avoid jeopardizing listed species.

A. *EPA will reserve (not promulgate) the proposed freshwater and saltwater acute and chronic aquatic life criteria for mercury in the final CTR.*

B. *EPA will promulgate a human health criterion of 50 ng/l or 51 ng/l as designated within the final CTR for mercury <u>only</u> where no more restrictive federally-approved water quality criteria are now in place (e.g., the promulgation will not affect portions of San Francisco Bay).*

C. *EPA will revise its recommended 304(a) human health criteria for mercury by January 2002. EPA will propose revised human health criteria for mercury in California by January 2003. These criteria should be sufficient to protect federally listed aquatic and aquatic-dependent wildlife species. EPA will work in close cooperation with the Services to evaluate the degree of protection afforded to federally listed species by the revised criteria. EPA will solicit public comment on the proposed criteria as part of its rulemaking process, and will take into account all available information, including the information contained in the Services' opinion, to ensure that the revised criteria will*

adequately protect federally listed species. If the revised criteria are less stringent than those proposed by the Services in the opinion, EPA will provide the Services with a biological evaluation/assessment on the revised criteria by the time of the proposal to allow the Services to complete a biological opinion on the proposed mercury criteria before promulgating final criteria. EPA will provide the Services with updates regarding the status of EPA's revision of the criterion and any draft biological evaluation/assessment associated with the revision. EPA will promulgate final criteria as soon as possible, but no later than 18 months, after proposal. EPA will continue to consult, under section 7 of ESA, with the Services on revisions to water quality standards contained in Basin Plans, submitted to EPA under CWA section 303, and affecting waters of California containing federally listed species and/or their habitats. EPA will annually submit to the Services a list of NPDES permits due for review to allow the Services to identify any potential for adverse effects on listed species and/or their habitats. EPA will coordinate with the Services on any permits that the Services identify as having potential for adverse effects on listed species and/or their habitat in accordance with procedures described in the draft MOA published in the Federal Register at 64 FR 2755 (January 15, 1999) or any modifications to those procedures agreed to in a finalized MOA.

D. EPA will utilize existing information to identify water bodies impaired by mercury in the State of California. Impaired is defined as water bodies for which fish or waterfowl consumption advisories exist or where water quality criteria necessary to protect federally listed species are not met. Pursuant to Section 303(d) of the CWA, EPA will work, in cooperation with the Services, and the State of California to promote and develop strategies to identify sources of mercury contamination to the impaired water bodies where federally listed species exist, and use existing authorities and resources to identify, promote, and implement measures to reduce mercury loading into their habitat. (See also "Other Actions B." below.)

E. EPA promulgated a new more sensitive analytical method for measuring mercury (see 40 CFR Part 136).

Services' assumptions regarding EPA's modifications to the proposed action for removing jeopardy.

In modifying our April 1998 jeopardy opinion and the modified draft RPAs considered in April 1999, the Services have assumed the following regarding EPA's proposed modifications:

Contaminant threats to listed species can be reduced through application of appropriately protective water quality criteria to the water bodies occupied by listed species.

The presumptive adverse effect threshold for identifying effects to listed species, is either the

exceedance of the criteria proposed in this opinion to protect listed species, or demonstrated effects below those proposed criteria concentrations for the priority pollutant under consideration.

The adjustments of criteria as proposed in the CTR by EPA for water bodies occupied by species considered in this opinion will be consistent with the effects analysis in this biological opinion unless new information is developed by EPA.

EPA adjustments of criteria will occur within agreed upon time frames.

Promulgations by EPA of the new mercury human health criterion will apply to all water bodies in California containing listed species and /or their habitats considered in this opinion by June of 2004.

The modification of 304a human health criterion for mercury which precedes EPA's promulgation of criteria in California will serve as the scientific guidance to permit writers for those permits with mercury discharges into waters occupied by listed species after January 2002

The revision of the human health mercury criterion will employ field derived bioaccumulation factors and this will result in a substantial lowering of the present criterion. The Services thus assume this revision will represent a substantial improvement statewide in the mercury water quality objectives for both listed aquatic species and wildlife species that forage within aquatic systems.

The draft CTR human health criterion of 51 ng/L will apply only where no more restrictive criteria are in effect, including San Francisco Bay.

The reservation of the acute and chronic aquatic life criteria for mercury means these criteria will not be used for regulatory purposes in California.

Pentachlorophenol (PCP)

Adequacy of Proposed Criteria

Aquatic Life Criteria

The EPA has proposed a pH-dependent freshwater acute criterion of 19 µg/L at pH= 7.8 (CMC = exp(1.005(pH)-4.830)), and a pH-dependent freshwater chronic criterion of 15 µg/L at pH=7.8 (CCC = exp(1.005(pH)-5.290)) for PCP (USEPA, 1997c). If the CTR is promulgated as proposed, salmonids and other listed fish could be exposed to ambient levels of PCP at or below the proposed acute and chronic criteria. After a review of the available data the Services conclude that the proposed acute and chronic water quality criteria for PCP are not protective of endangered and threatened fish. Current literature indicates adverse effects of commercial (technical grade) PCP on reproduction, early life stage survival, growth, or behavior of salmonid

species at concentrations at or below the proposed criteria. EPA has not included within the criteria interactive effects of pH, dissolved oxygen or temperature on toxicity of PCP to fish. These factors exacerbate the deleterious effect of PCP toxicity on salmonids at the proposed criteria concentrations. The criteria also do not consider bioconcentration of PCP or its impurities into aquatic organisms and subsequent ingestion by wildlife.

EPA has suggested to the Services that drinking water standards for PCP (0.28 µg/L) could serve to protect salmonids. These standards, however, do not apply in water bodies without the appropriate MUN designation. MUN is the beneficial use designation for water bodies that serve as municipal and domestic water supply. The following water bodies serve as habitat for listed fish species and do not have the MUN designation. Listed salmonids and other fish species in these water bodies are dependent upon the aquatic life criterion alone for protection. Therefore, adverse effects to listed species occurring within these water bodies are likely to occur.

Region 1:	Laguna de Santa Rosa
Region 2:	First Valley Creek (tributary to Drake's Estero)
	Coast Creek
	Alamere Creek
	Bolinas Bay tributaries
	Rodeo Creek (tributary to Rodeo Lagoon)
	Millerton Gulch (tributary to Tomales Bay)
	Walker Creek and tributaries
	Bear Valley Cr., Devil's Gulch, and Gulch Creek (tributaries to Olema Creek)
	Frenchman's Creek
	Purisima Creek
	Lobitas Creek
	Tunitas Creek
	San Gregorio Creek and tributaries
	Pomponio Creek
	Butano Creek
	San Rafael Creek
	Corte Madera Creek and tributaries
	Coyote Cr., Old Mill Cr., and Arroyo Corte Madera del Presidio (tributaries to Richardson Bay)
	San Leandro Creek and tributaries
	Alameda Creek and tributaries
Region 3:	Watsonville Slough and tributary sloughs
Region 5:	Battle Creek
	Thomes Creek
	Big Chico Creek
	Stony Creek
	Butte Creek (below Chico)

Lower Yuba River (below Engelbright Dam to Feather River)
Mokelumne River (Comanche Reservoir to Delta)

Hazards of PCP

PCP Sources, Chemistry, and Environmental Fate

PCP at one time, was one of the most widely used biocides. In 1986, approximately 28 million pounds were used in the United States. It was registered for use as a molluscide, fungicide, herbicide, insecticide, disinfectant, wood preservative, slimicide in pulp and paper products, and paint preservative. Its use was restricted by EPA since 1984, consequently it is no longer available for home and garden use (ATSDR 1993). Approximately 80% of the total technical grade PCP use is for wood preservation. The majority of wood treated with PCP is done so commercially, using pressurized treatment. Treatment with PCP results in a 5 to 8-fold increased useful life of wood products. The aqueous form, sodium pentachlorophenate (NaPCP) has been used in pressboard, insulation, and industrial cooling water, among other uses (Crosby 1981; Eisler 1989).

In the U.S., PCP is produced by the chlorination of phenols in the presence of catalysts. The alternative production process, hexachlorobenzene hydrolysis, is not used in the U.S. Commercial grades of pentachlorophenol, also referred to as technical PCP, are generally about 86% pure. Reagent grade and purified forms of PCP have been used extensively in toxicity testing in order to differentiate the toxicity of PCP in relationship to the numerous impurities found in commercial preparations. However, the Services assume that technical grades of PCP are the forms more commonly released to the environment.

Impurities found in commercial preparations of PCP include relatively high concentrations of chlorophenols, polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), hexachlorobenzene, chlorinated phenoxyphenols, and chlorinated diphenyloxides (USEPA 1980; Eisler1989; Cleveland *et al*. 1982; Hamilton *et al*. 1986). Chlorinated phenoxyphenols and other compounds found in PCP can be precursors to the formation of PCDD/Fs (Cleveland *et al*. 1982; Hamilton *et al*. 1986). PCDD/Fs are known to bioaccumulate in the environment and are also highly toxic to avian and mammalian wildlife. The bioaccumulation and chronic toxicity to wildlife of the other impurities found in commercial PCPs are not well known. The commercial preparations of PCP have been found to be 5 to 6-fold more toxic to fathead minnow than are purified PCP forms. It is believed that the impurities in commercial PCPs are largely responsible for the enhanced toxicity (Cleveland *et al*. 1982).

PCP can be released into the aquatic environment in runoff and in wood-treatment effluents. The majority of wood treatment plants evaporate their waste water, so they do not discharge to surface waters. The rest of the wood treatment plants discharge to waste-water treatment facilities. Prior to EPA restricting its use, discharges to water totaled approximately 37,000 pounds annually. Releases to the aquatic environment now are expected to be less. In 1991, Toxics Release

Inventory data indicates total releases to the environment (including discharge to water, air and soil) from certain facilities were 16,296 pounds. Total releases to the environment are likely higher than reported by the Toxic Release Inventory, because data are available for only certain types of facilities required to report releases (ATSDR 1993).

PCP is soluble in most solvents, and slightly soluble in water. In contrast, the sodium salt of PCP, NaPCP, is very water soluble. However, the chemical properties of PCP are closely related to the pH of the aqueous solution. PCP has a pK_A of 4.7, which means that at a pH of 4.7, aqueous solutions will contain 50% ionized PCP. At pH 6.7, in the range of many natural waters, PCP is 99% ionized. However the toxicity of PCP increases as the pH of the water decreases, because the un-ionized form (which is favored at low pH) passively diffuses across the gill membrane (USEPA 1986). The proposed criteria are pH-dependent because PCP ionization in water increases with an increase in pH (i.e., PCP is more toxic at lower pH because the un-ionized form which crosses the membrane is predominant over the ionized form).

Once released to water, the half-life of PCP ranges from less than one day to 15 days. The degree of degradation is controlled by amount of incident radiation (sunlight penetration), dissolved oxygen, and pH of the water. Photolysis and degradation by microorganisms are considered the major mechanisms by which PCP is degraded in water. Degradation of PCP in water forms other compounds, primarily pentachloroanisole, 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol, and 2,3,5,6-tetrachlorophenol (ATSDR 1993).

Ambient surface water concentrations of PCP have been reported to generally be between 0.1 to 10 µg/L (as of 1979, ATSDR 1993). These values are within the range of the proposed chronic criterion for PCP (assuming a neutral pH = 6.7, the chronic criterion is 4.95 µg/L). Industrialized areas, and areas near paper mills and wood treatment facilities, have levels at the high end of that range, or even higher. However, much of the existing published data on surface water concentrations is from the 1970's, prior to its use restrictions by EPA. Collecting additional data on ambient PCP concentrations in streams supporting federally listed fish would help identify locations where PCP may be a problem for listed fish species.

Toxicity

The mechanism of PCP toxic action is regarded to be via reduced production of adenosine triphosphate (ATP) and alteration of liver enzymes, which control energy metabolism. The response to this effect is an increased basal metabolism, resulting in increased oxygen consumption and high fat utilization (Webb and Brett 1973; Chapman and Shumway 1978; Johansen *et al.* 1985; Nagler *et al.* 1986; Eisler 1989). Growth parameters and locomotion/activity have been found to be sensitive endpoints for salmonids and other fish exposed to PCP (Hodson and Blunt 1981; Webb and Brett 1973; Dominquez and Chapman 1984; Brown *et al.* 1985; Johansen *et al.* 1987; Brown *et al.* 1987). The fact that the mechanism of action affects energy metabolism is support for use of growth parameters (e.g., reduced growth rate, reduced biomass) and activity parameters (reduced swimming activity, reduced prey

consumption, reduced predator avoidance) to be used as sensitive and appropriate endpoints in sublethal toxicity tests. This mechanism also supports the conclusion that early fry, which have just finished utilizing the yolk sac and have begun to feed on exogenous sources of food, are among the most sensitive life stages tested.

In general, fish are more sensitive to PCP than are other aquatic organisms. Salmonids have been found to be the most sensitive fish species tested under acute exposure conditions (Choudhury *et al*. 1986; Eisler 1989; USEPA 1980, 1986b, 1995b, 1996c). Warmwater species are generally less sensitive than coldwater species in acute lethal toxicity tests (USEPA 1995c). Evaluation of threatened or endangered salmonid species against the rainbow trout, a typical test organism, found that the Apache trout (*Oncorhynchus apache*) was more sensitive than the rainbow trout in acute lethality tests with PCP, indicating an additional margin of safety may be needed to protect listed salmonids when using rainbow trout test data in toxicity assessments (USEPA 1995c). EPA (1995) also recommends that "further testing be done on listed species or their FWS-identified surrogate species before definitive policy decisions concerning the protection of endangered and threatened species are made. In addition, chronic toxicity assessments should be conducted in order to compare chronic responses between listed and surrogate species."

Early life stage of salmonids, such as sac fry and early fry, have been found to be more sensitive than later life stages and even more sensitive than embryos, to acute exposures of PCP. Similarly, early life stage of largemouth bass have varying sensitivity to acute exposures of PCP (Johansen 1985). Acute toxicity of PCP to fathead minnow also varies with life-stage, but adults appear to be more sensitive than juveniles or fry to PCP (Hedtke *et al*. 1986). In a study by Adema and Vink (1981) 96-hour lethal concentrations for 50 percent of the populations tested (LC_{50}s) in guppy ranged between 450 to 1,600 µg/L (life stage only specified as young or adult). Early life stages of the plaice (*Pieuronectes platessa*) were more sensitive with 96-hour LC_{50}s ranging from 60 to 750 µg/L at pH of 8; the larval stage was the most sensitive and the egg the least sensitive of the life stages tested. LC_{50}s for early life stage salmonids are lower at between 18 to 160 µg/L (Table 8a). Thus, non-salmonid fish appear to be less sensitive at early life stages than salmonids to acute toxicity of PCP.

Summary of Effects of PCP on Listed Species

Salmonids

Salmonid species evaluated include: all ESUs and runs of listed or proposed coho and chinook salmon and steelhead trout, Lahontan cutthroat trout, Paiute cutthroat trout, and Little Kern golden trout.

Tables 8a and 8b summarize the critical acute and chronic studies conducted on salmonid species used in this analysis. The proposed EPA criteria are dependent upon pH. To compare the water concentrations of PCP used in the study to the criteria, the final column in Tables 8a and 8b derives an acute and chronic water quality criterion using equations described in USEPA (1995b)

for the pH at which the study was conducted. (There appears to be an error in footnote "f" in the Federal Register table. We based our pH corrections on the pH-dependent equations listed on pp. M-1, M-2 of USEPA 1995b).

Acute Studies: The first study listed in Table 8a is an acute study on rainbow trout conducted by Little et al. (1990). These researchers evaluated behavioral effects with implications for survival in the environment. Chapman's review of the draft biological opinion criticized this study stating that the acetone could artificially enhance uptake of PCP impurities (Chapman 1998). Although this may occur no studies have been done to evaluate the hypothesis. Since acetone was also in the control group, the effects of acetone itself is not at issue. Chapman (1998) goes on to recommend that proper studies be done to resolve the issues regarding differences in toxicity between commercial PCPs and the purified forms of PCP. Another limitation of the Little et al. (1990) study is that only nominal concentrations of PCP in test water are reported; water samples do not appear to have been analyzed to confirm the test concentrations. The evaluated behaviors of the Little *et al*. (1990) study included swimming activity, swimming capacity, feeding, and vulnerability as prey. Swimming capacity was not affected. Survival from predation did not show a clear dose-response curve; greater survival was observed in the 2 µg/L compared to the 0.2 µg/L group. Similarly, there was not a clear dose-response for number of prey consumed and swimming activity. There was significantly reduced swimming activity and prey consumption observed at 2 µg/L of technical grade PCP after 4 days of exposure, compared to controls. As Chapman (1998) points out, determining safe levels from this study is difficult given the experimental design and the lack of clear dose-response for many of the endpoints evaluated. Also, Chapman (1998) indicates that this study does not report whether pH was monitored during the tests. However, even if the pH of the static test solutions were a full pH unit lower than measured in the well water (i.e., pH = 6.8 instead of 7.8), the acute criterion of 7.13 µg/L and the chronic criterion of 5.47 µg/L (at pH = 6.8) would still be greater than the concentrations at which effects on behavior were observed. Therefore, the proposed acute criterion for PCP of about 19.5 µg/L (pH-adjusted to pH = 7.8) is not protective of salmonid behavior relative to growth and survival.

Table 8a: Summary of Critical Acute Studies on the Effects of PCP in Salmonids.

Citation	Life Stage and Species#	Exposure Duration, days	Test Solution	Test Type	Effect	pH	Effect concentration, µg/L	pH Adjusted Criteria*, µg/L
Little et al. (1990)	0.5 - 1.0 g *O. mykiss*	4	Tech. grade PCP	static	reduced swimming activity and reduced prey consumption	7.8	LOAEL = 2 NOAEL = 0.2	19.5
Van Leeuwen et al. (1985)	early fry (77 days) *O. mykiss*	4	97 percent purified PCP	static renewal	50 percent mortality	7.2	18 (96 hr. LC_{50})	10.6
Van Leeuwen et al. (1985)	sac fry (42 days) *O. mykiss*	4	97percent purified PCP	static renewal	50 percent mortality	7.2	32 (96 hr. LC_{50})	10.6
Dominguez and Chapman (1984)	fry (70 days) *O. mykiss*	4	99 percent purified PCP	flow-through	50 percent mortality	7.4	66 (96 hr LC_{50})	13
Davis & Hoos (1975)	1-3 g *O. mykiss*	4	NaPCP	static	50 percent mortality	5.7 - 7.0	45 - 100 (96 hr LC_{50})	2.3 - 8.7
Davis & Hoos (1975)	1-3 g *O. kisutch*	4	NaPCP	static	50 percent mortality	7.0	32 - 96 (96 hr LC_{50})	8.7
Davis & Hoos (1975)	1-3 g *O. nerka*	4	NaPCP	static	50 percent mortality	7.2 - 7.7	50 - 130 (96 hr LC_{50})	10.6 - 17.6
U.S. FWS (1986)	0.3g fry *O. tshawytscha*	4	96 percent Technical Grade PCP	static	50 percent mortality	7.4	31 (96 hr LC_{50})	13
U.S. FWS (1986)	1.0g fry *O. tshawytscha*	4	96 percent Technical Grade PCP	static	50 percent mortality	7.4	68 (96 hr LC_{50})	13
U.S. FWS (1986)	yolk-sac fry *O. mykiss*	4	96 percent Technical Grade PCP	static	50 percent mortality	7.4	121 (96 hr LC_{50})	13
U.S. FWS (1986)	1.0g fry *O. mykiss*	4	96 percent Technical Grade PCP	static	50 percent mortality	7.4	34 - 52 (96 hr LC_{50})	13
U.S. FWS (1986)	1.0g fry *O. mykiss*	4	NaPCP	static	50 percent mortality	7.4	55 - 58 (96 hr LC_{50})	13

Citation	Life Stage and Species#	Exposure Duration, days	Test Solution	Test Type	Effect	pH	Effect concentration, µg/L	pH Adjusted Criteria*, µg/L
U.S. FWS (1986)	yolk-sac fry *O. mykiss*	4	NaPCP	flow-through	50 percent mortality	7.4	160 (96 hr LC_{50})	13
U.S. FWS (1986)	swim-up fry *O. mykiss*	4	NaPCP	flow-though	50 percent mortality	7.4	165 (96 hr LC_{50})	13
U.S. FWS (1986)	eyed-egg *O. mykiss*	4	NaPCP	flow-through	50 percent mortality	7.4	>300 (96 hr LC_{50})	13
U.S. FWS (1986)	0.3g fry *O. tshawytscha*	4	NaPCP	flow though	50 percent mortality	7.4	165 (96 hr LC_{50})	13
U.S. FWS (1986)	swim-up fry *O. tshawytscha*	4	NaPCP	flow-through	50 percent mortality	7.4	>250 (96 hr LC_{50})	13
U.S. FWS (1986)	1.0g fry *O. tshawytscha*	4	NaPCP	static	50 percent mortality	7.4	67.5 (96 hr LC_{50})	13
U.S. FWS (1986)	yolk-sac fry *O. tshawytscha*	4	NaPCP	static	50 percent mortality	7.4	30.5 (96 hr LC_{50})	13
U.S. EPA (1995)	0.5 - 1.0g fry *O. mykiss*	4	99 percent purified PCP	static	50 percent mortality	8.2	160 (96 hr LC_{50})	30
U.S. EPA (1995)	0.5 - 1.0g fry *O. apache*	4	99 percent purified PCP	static	50 percent mortality	8.2	110 (96 hr LC_{50})	30
U.S. EPA (1995)	0.5 - 1.0 fry *O. clarki stomias*	4	99 percent purified PCP	static	50 percent mortality	8.2	>10 (96 hr LC_{50})	30
U.S. EPA (1995)	0.5 - 1.0 fry *O. clarki henshawi*	4	99 percent purified PCP	static	50 percent mortality	8.2	170 (96 hr LC_{50})	30

* acute criterion (µg/L) = $e^{(1.005\ (pH)\ -\ 4.869)}$

\# *O. mykiss* = rainbow trout
O. apache = Apache trout
O. clarki stomias = Greenback cutthroat trout
O. clarki henshawi = Lahontan cutthroat trout
O. kisutch = Coho salmon
O. nerka - sockeye salmon

O. tshawytscha = Chinook salmon

Table 8b: Summary of Critical Chronic Studies on the Effects of PCP in Salmonids

Citation	Life Stage and Species#	Exposure Duration, days	Test Solution	Test Type	Effect	pH	Effect concentration, µg/L	pH Adjusted Criteria*, µg/L
Dominguez and Chapman (1984)	egg through day 72 *O. mykiss*	72	99 percent purified PCP	flow-through	34 percent mortality; decreased weight and length; increased fin erosion and mild malformations	7.4	19	10
Dominguez and Chapman (1984)	egg through day 72 *O. mykiss*	72	99percent purified PCP	flow-through	NOAEL for mortality, growth	7.4	11	10
Chapman and Shumway (1978)	fertilization of egg through complete yolk absorption *O. mykiss*	chronic	Tech. grade Na PCP	flow-through	little or no mortality compared to control at D.O. = 10 mg/L	7.8	10	15
Chapman	''	chronic	Tech. grade	flow-through	27.4 percent	7.8	10	15
Chapman and Shumway (1978)	''	chronic	Tech. grade Na PCP	flow-through	100 percent mortality at D.O. = 3 mg/L	7.8	10	15
Chapman (1969)	alevin *O. mykiss*	20-35	Tech. grade Na PCP	flow-through	15% reduction in weight gain	7.8?	30	15
Webb and Brett (1973)	subyearling *O. nerka*	14 - 56 (+ 4 weeks post-exposure exam)	Na PCP	flow-through	growth rate and food conversion efficiency	6.8	EC50 for growth rate = 1.74 EC50 for conversion efficiency = 1.8	5.5
Matida *et al.* (1971)	fry (2.1 - 2.5 g) *O. mykiss*	28	Tech. grade Na PCP	flow-through	27 percent growth inhibition	7.2	8	8.2
Nagler et al. (1986)	adult female *O. mykiss*	18	99 percent purified PCP	flow-through	reduced number of viable oocytes	7.5	LOAEL = 21.8 NOAEL = 11.5	11

Citation	Life Stage and Species#	Exposure Duration, days	Test Solution	Test Type	Effect	pH	Effect concentration, µg/L	pH Adjusted Criteria*, µg/L
Iwama et al. (1986)	juvenile (15 g) *O. tshawytscha*	40	Na PCP	flow-through	changed in blood BUN and GLU	?	3.9	15 @ pH = 7.8
Hodson and Blunt (1981)	embryo and alevin (after hatch to early fry) *O. mykiss*	exposed from embryo or alevin through fry feeding for 4 weeks	99percent purified NaPCP	flow-through	reduced wet weight, growth rate, and biomass at 20°C	7.78 - 8.08	11-16	18.2 @ pH = 8.0

F. chronic criterion (µg/L) = $e^{(1.005(pH) - 5.134)}$ (USEPA 1995b)

O. mykiss = rainbow trout
O. nerka = sockeye salmon
O. tshawytscha = Chinook salmon

One of the more comprehensive papers on the lethal effects of PCP on salmonids described a series of acute toxicity tests conducted on a range of early life stage rainbow trout (Van Leeuwen *et al.* 1985). LC_{50} 96-hour values for six early life stages (from egg through early fry) were determined. LC_{50} values ranged over 167 fold, with eggs being the least sensitive and early fry, the most sensitive life stages. Table 8a lists the LC_{50} value of 18 µg/L for the most sensitive life stage tested, early fry. The second most sensitive life stage was sac fry, with an LC_{50} of 32 µg/L. Van Leeuwen *et al.* did not develop a NOAEL for these life stages, so we cannot assess whether the proposed chronic criterion of 8.2 µg/L and acute criterion of 10.6 µg/L (adjusted for pH) would be protective against significant mortality of sensitive early life stage salmonids. As Chapman (1998) indicates, one problem with this study design is that acetone, which may or may not enhance toxicity of impurities in PCP, was used in the test groups but not in the control. Chapman (1998) also notes another flaw of this study is that pH was not monitored, so it is unclear what the pH was during the test. Nonetheless, the Van Leeuwen *et al.* (1985) study indicates the relative sensitivities in mortality between various early life stage of salmonids due to short-term exposures of PCP.

There are differences in the 96-hour LC_{50} calculated for early life stage salmonids between the Van Leeuwen *et al.* (LC_{50} = 18 µg/L) and the Dominquez and Chapman (66 µg/L) studies. The early fry stage (approximately 77 days), found to be the most sensitive in the Van Leeuwen study, appears to have been tested in the Dominquez and Chapman study. Chapman (1998) maintains that the fry used in their study were "probably farther advanced" than the developmental stage of the fry found to be most sensitive in the Van Leeuwen *et al.* study; this contention is difficult to verify given that neither Van Leeuwen et al (1985) or Dominquez and Chapman (1984) provide specific information on state of yolk sac absorption in the fry tested, and the studies test different forms of the same species (anadromous steelhead versus rainbow trout). Chapman (1998) suggests that factors responsible for the differences in LC_{50} s include the use of acetone as a carrier in the Van Leeuwen *et al.* study, or differences in pH not measured in the Van Leeuwen study. Other experimental design differences between the two studies include: static renewal versus flow-through design, differences in purity of the PCP compound , and variety of salmonid (steelhead versus rainbow trout). Nevertheless, the essential point is that both studies indicate that PCP causes significant lethality in early life stage salmonids after exposures as short as 4 days. The narrow range between the proposed acute and chronic criteria is insufficient to protect early life stage, since the chronic criterion is a four-day average concentration limit which is also the duration of these acute studies. There is only a 2-fold difference between the chronic criterion and the LC_{50} for early fry determined by Van Leeuwen *et al.* (1985) (8.2 versus 18 µg/L). There is only a 6-fold difference between the chronic criterion and the LC_{50} for fry determined by Dominquez and Chapman (1984) (10 versus 66 µg/L). Since the LC_{50} is the concentration at which half of the organisms die, both these studies suggest it is likely that some mortality would occur at PCP concentrations at or below the proposed chronic criterion.

An interlaboratory bioassay testing program was conducted using rainbow trout, coho salmon, and sockeye salmon (Davis and Hoos 1975). The pH of the test water varied with lab, as did the LC_{50} values which ranged from 37 µg/L to 130 µg/L sodium pentachlorophenate. No apparent species

sensitivity in acute lethality was observed, and the authors concluded that any major variation in toxicity value were explained by physical and chemical characteristics of the bioassay (pH, water temperature, etc.)

The U.S. Fish and Wildlife Service (USFWS 1986) conducted a series of acute bioassays using technical grade PCP and the sodium salt (Na PCP), on various life stages of chinook salmon and rainbow trout. The results of these studies indicate that swim-up, sac fry and eyed embryos of chinook and rainbow trout are less sensitive than the 1.0 g- size fry to the acute exposures of both technical grade PCP and NaPCP. The lowest LC_{50} was for a 0.3 g chinook salmon: the 0.3 g fry was twice as sensitive as the 1.0 g fry (LC_{50} s of 31 µg/L vs. 68 µg/L technical grade PCP). For 1.0 g fry, chinook were somewhat less sensitive than rainbow trout to technical grade PCP (LC_{50} s of 68 µg/L and 34 to 52 µg/L, respectively). Similarly for NaPCP, chinook fry were somewhat less sensitive than rainbow trout (LC_{50} s of 67 µg/l and 55 to 58 µg/L respectively). It is interesting to note that the 24-hour LC_{50} values for 1.0 g-size fry are very close, or identical to, the 96-hour LC_{50}. This suggests that short-term exposures of PCP to ELS salmonids are as detrimental as 4-day exposures. In other words, the exposure time for mortality to occur is very short.

A series of acute lethality studies on salmonids (USEPA 1995c) evaluated three different listed salmonid species against the rainbow trout. This study found that there were species differences in sensitivity under acute exposures, with the Apache trout being more sensitive than the other species tested. The 96-hour LC_{50} s from these studies were higher by a factor between 3 to 9 than the other acute studies listed in Table 8a. During the test, there was a variation in pH, and some of the test runs had dissolved oxygen levels below 60% saturation at 48 hours or below 40% saturation at 96 hours. USEPA (1985) found that there was no apparent trend in results for test with varying water quality, and did not eliminate any tests or modify calculation of LC_{50} s. As was found in the USFWS (1986) studies, the 24-hour LC_{50} s were close to the 96-hour LC_{50}, indicating the exposure time for mortality to occur is very short. USEPA (1995) concluded , "Further [acute] testing should be conducted with other listed species or their FWS-identified surrogate species before definitive policy decisions concerning the protection of endangered and threatened species are made".

To summarize the various acute lethality studies conducted on ELS salmonids, the LC_{50} s on rainbow trout fry (0.5 to 1.0 g) using technical grade PCP (USFWS 1986) were lower than similar studies using purified PCP (Dominguez and Chapman 1984). The results of the Van Leeuwen et al (1985) on 97 percent purified PCP had the lowest LC_{50} of 18 µg/L. The studies conducted by USEPA (1995) on acute lethality of similar -size rainbow trout fry were from 3 to 9 times higher (indicating less sensitivity) than either of the previous studies. The 96-hour LC_{50} s for early fry rainbow trout (which appears to be one of the most sensitive life stages) varies between 18 to 160 µg/L, or almost an order of magnitude. Factors that may contribute to the variation in LC_{50} values include differences in form of PCP tested and the pH of the test solution.

As Table 8a indicates, the acute criterion at the pH of the test solution is below the LC_{50} value.

However, by definition the LC_{50} is the concentration at which half of the organisms are expected to die, and cannot be used to determine the concentration that would be lethal to low numbers of salmonid trout exposed for a short period of time. Therefore, due to the uncertainty as to the true LC_{50} for ELS salmonids using commercial grades of PCP, there is an apparent need for EPA to conduct additional acute bioassays. Also, due to the uncertainty as to the true LOAEL and NOAEL for sublethal effects for ELS salmonids using commercial grades of PCP under acute exposures, there is an apparent need for EPA to conduct additional acute bioassays using sensitive sublethal endpoints.

Chronic Studies: Chronic studies are summarized in Table 8b. A chronic exposure study on early life stage salmonids was conducted by Dominguez and Chapman (1984) using purified PCP instead of commercial grade PCP. They exposed rainbow trout from the embryo stage through 72 days of development. Dominguez and Chapman found 34 percent mortality at 19 µg/L PCP at the end of the test. A significant reduction in weight of the trout at 19 µg/L PCP was observed compared to controls (32% reduction in weight). At 11 µg/L PCP level, weight was reduced 15% compared to controls, but was not statistically significant. Other effects observed included increased fin erosion, mild malformations, and lethargy. A NOAEL for mortality of 11 µg/L was also determined. The pH-adjusted chronic criterion would be 10 µg/L, which is essentially the same as the acute NOAEL. One limitation of the Dominquez and Chapman study is that only nominal concentrations of PCP in test water are reported; water samples do not appear to have been analyzed to confirm the test concentrations. Another limitation with this study is that purified PCP, not commercial PCP was used in the test. As discussed in more detail below, purified PCP formulations are believed to be less toxic than commercial PCP formulations. Therefore, the Dominquez and Chapman (1984) NOAEL of 11 µg/L using purified PCP suggests that the chronic criterion of 10 µg/L at pH =7.4 would not be protective of salmonids exposed to commercial forms of PCP.

Early work by Chapman (1969) found an average of 15% reduced weight gain compared to controls in alevins (sac-fry) exposed to 30 µg/L PCP for between 20 and 35 days at 10 and 15 °C. Juvenile steelhead had a 17% reduction in weight gain compared to controls after a 3 week exposure to 30 µg/L PCP. A NOAEL could not be determined from these experiments because 30 µg/L was the lowest concentration tested and because Chapman did not statistically evaluate the data for differences. Chapman (1969) concludes that alevin growth decreased by 6% for each 10 µg/L increase in PCP. These observed effects on growth in both sac-fry and juvenile salmonids after a few weeks of exposure indicate that growth is a sensitive sublethal endpoint for early life stage salmonids.

In a study using young-of-the-year sockeye salmon, Webb and Brett (1973) derived median effect concentrations for growth rate and food conversion efficiency. The EC50 for growth effects was calculated to be 1.74 µg/L, and for food conversion efficiency was calculated as 1.8 µg/L (Webb and Brett 1973). This concentration was approximately 2.8 percent of the 96-hour LC 50. Chapman (1998) notes that the graphical techniques used by Webb and Brett provide a best estimate of an effect-no effect threshold concentration, and not an EC50 as is commonly

interpreted (the concentration at which 50 percent of the organisms are expected to exhibit the sublethal response). The study design also varied the exposure duration for different test concentrations, making comparisons between various test concentrations and controls difficult. The control and 3.42 µg/L PCP exposure had the same exposure duration of 56 days; a 10% reduction in growth was observed at that concentration compared to controls. Whether that level of reduced growth was statistically significant was not determined by the authors. Effects on growth rate and conversion efficiency continued post-exposure at greater than 2 µg/L PCP, although some recovery from effects was observed. Swimming performance was not affected in this test, leading these researchers to conclude that growth responses are more sensitive indicators than swimming. Chapman (1998) criticized this study as being unrealistic because the flowrate of 20 cm/sec during the tests may have unrealistically increased the energy demands of the fish, making them more sensitive than usual to the effects of PCP. However, Webb and Brett (1973) concluded that feeding and assimilation efficiency were unaffected by PCP, which implies that unusual energy demands were not placed on the fish at the flowrate of the study. Additionally, 20 cm/sec is within the range of swimming speeds reported for underyearling coho salmon of 6 to 30 cm/second (Sandercock 1991). Since the observed effects were seen during PCP exposure, in contrast to a control group that also experienced the same flowrate, the Services conclude that this study is relevant.

In a study by Matida *et al.* (1970), rainbow trout fry were exposed to 3, 8 and 20 µg/L PCP for 28 days. At 20 µg/L PCP mortality was greater than in the controls (13.3% vs. 3.3%), and there was decreased weight gain compared to controls (39.7% versus 98.3%). At 8 µg/L PCP, mortality also appeared elevated compared to controls (16.7% versus 3.3%), and weight gain was apparently decreased (70.4% versus 98.3%). At 3 µg/L PCP, mortality was elevated compared to controls (16.7% versus 3.3%), and weight gain was decreased slightly (92.8% versus 98.3%). Use of this study to set criteria is problematic because the study design did not allow for evaluating the statistical significance of the results, and it does not appear that pH was measured during the test. There appears to be a dose-response to PCP for weight gain, but not for mortality. This study, along with the study by Webb and Brett (1973) indicate that growth is a more sensitive endpoint than mortality for young salmonids, and that effects on growth occur at concentrations at or below the proposed chronic criterion.

One of the few studies to date on reproductive effects in adult salmonids was conducted by Nagler *et al.* (1986). This study revealed adverse impacts on ovarian development at 22 µg/L after an 18-day exposure. Effects on ovarian development were not seen at 11 µg/L, the adjusted chronic criterion (rounded). However, this study was conducted on purified PCP, not technical grade PCP, the formulation released into the environment. Cleveland *et al.* (1982) demonstrated that contaminants in technical grade PCP increased the sublethal toxicity to fathead minnow by a factor of 6 compared to purified PCP. Therefore, it has not been shown that the proposed chronic criterion would be protective against reproductive effects in adult salmonids chronically exposed to technical grade PCP. PCP has been shown to affect reproduction in adult salmonids, as well as having lethal and sublethal effects on early life stage salmonids. The cumulative effect of both reduced reproductive success in adults along with reduced survival or fitness of young, is not

addressed by the proposed chronic criterion.

It has been established that commercial PCPs are significantly more toxic to aquatic organisms than are the purified forms of PCP (Cleveland *et al*. 1982; Eisler 1989). Chapman (1998) criticizes the Cleveland *et al*. 1982 study, which demonstrated that the commercial PCP was more toxic than purified forms to fathead minnow in a partial life-cycle test, because small amounts of acetone were used to solubilize the PCP. However, as previously stated, no studies have been performed to confirm this hypothesis. Chapman (1998) cites his own work as not indicating a difference in toxicity between pure and technical grade PCP. However, in the Dominquez and Chapman (1984) study, fry that were past yolk sac absorption and exogenous feeding were exposed to purified PCP, while Chapman (1969) exposed fry to commercial PCP prior to onset of exogenous feeding. Thus, the differences in life-stage tested between the two studies confounds the interpretation of toxicity due to either purified or commercial PCP. Chapman (1998) suggests that technical grade PCP can vary in the nature and toxicity of impurities, and proposes using Whole Effluent Toxicity (WET) testing as a regulatory option for discharges of PCP. Therefore, there is a need for EPA to evaluate using WET in permitted discharges. However, WET would be less useful for evaluating non-point sources of commercial PCPs in the environment, or in establishing ambient water quality criteria.

In summary, the papers cited above indicate that the proposed chronic criterion for PCP would not be protective against lethal or sublethal effects on early life stage salmonid species. Because of the effects on adult reproduction, and effects on early life stage salmonids observed at concentrations at or below the proposed chronic criterion, there is an apparent need for EPA to conduct critical life-cycle tests on salmonids in a manner which meets their requirements for deriving a chronic value, using commercial preparations of PCP. Such tests should include the effects of pH, elevated temperatures, and low dissolved oxygen on lethal and sublethal effects to salmonids, and should include sensitive endpoints such as growth and behavior.

Chapman (1998) concludes, "Overall, the Services are justifiably concerned that the current EPA criterion for PCP might not be sufficiently conservative to provide protection for endangered species of salmonid fish and perhaps other nonsalmonid species. It appears that the most defensible means of providing this protection is to use a more conservative acute-to-chronic ratio and include further protection to account for expected conditions of dissolved oxygen reduction and/or temperature elevation." Chapman (1998) also reviews the literature and the acute-to-chronic ratio used by EPA and concludes, " The Services' comments regarding the EPA's derivation of an acute-to-chronic ratio are apt. I agree with their finding that a larger ACR [acute-to-chronic ratio] is suggested by the available data." Chapman derives an acute-to-chronic ratio for the protection of fish species of 5.219 for PCP (in contrast to an acute-to-chronic ratio of 2.608 cited in USEPA 1995b). Therefore, there is an apparent need for EPA to re-evaluate the basis for the acute-to-chronic ratio.

Cumulative and Interactive Effects: Another study on early life stage steelhead trout, conducted by Chapman and Shumway (1978), examined the effects of low dissolved oxygen in conjunction with PCP exposure. These researchers found significant mortality in early life stage salmonids at 10 µg/L PCP under low dissolved oxygen conditions. This study indicates the importance of other water quality parameters in addition to pH in establishing water quality criteria. Chapman (1998) concludes that the Chapman (1969) and Chapman and Shumway (1978) studies "probably understate the effects that would be observed in a true early life stage study." Thus, exposure to the chronic criterion for PCP is likely to result in increased mortality of early life stage salmonids under low dissolved oxygen conditions.

A study on juvenile chinook salmon was conducted by Iwama *et al*. (1986). Chronic exposure to 3.9 µg/L resulted in alteration of blood chemistry parameters (blood urea nitrogen (BUN) and glucose (GLU)). As noted by Chapman (1998), the significance of the altered blood chemistry is uncertain as to impacts on growth, survival and behavior. However, Iwama *et al*. (1986) indicate that these altered blood chemistry are indicative of hyperglycemia and suggest the effect is due to the stress of PCP exposure, though they do not rule out handling as a possible factor causing the stress. The altered blood chemistry is further evidence that adverse biochemical effects on salmonids may occur at levels below the proposed chronic criterion. Results of this study also suggest, but are not conclusive, that there may be an interaction between infectious agents and PCP in the concentration range of the proposed water quality criteria, with PCP exposure possibly enhancing the effects on infected fish. No changes in feeding or schooling behavior were observed at either test concentration.

Hodson and Blunt (1981) investigated the interactive effects of PCP and temperature on early life stages of rainbow trout. The study found that at 20°C, biomass of fish exposed to 11 to 16 µg/L NaPCP was reduced compared to controls. Reduced biomass, wet weight, and growth rate were observed both for fish exposed as embryos and for fish exposed at day of hatch, through 4 weeks of feeding as fry. In contrast, under a colder temperature regime (10°C), biomass of early life stage was not reduced until PCP concentrations were greater than 20 µg/L. At PCP concentrations greater than 20 µg/L (10°C), mortality of embryos and larvae, delayed hatching and reduced yolk sac resorption efficiency were observed, in addition to effects on biomass and growth rate. Hodson and Blunt also observed that early life stage salmonids exposed from fertilization were more sensitive to the effects of PCP than salmonids exposed only after hatch. Mortality of early life stage was determined to be a function of PCP concentration, temperature, and life-stage exposed. Effects on growth rate of early life stage were a function of PCP concentration and temperature, but not the life-stage exposed. Thus, this study demonstrates that temperature and life-stage are important considerations in developing a chronic criterion for PCP, in addition to pH. This study indicates that in warm water environments the proposed chronic criterion would not be protective of salmonids to sublethal effects of reduced growth rate and weight.

In summary, the proposed chronic criterion does not address the cumulative and interactive effects of PCP toxicity through the critical life-cycle, or under conditions of elevated temperatures or

reduced dissolved oxygen. There is an apparent need for EPA to revise the proposed chronic criterion to address the cumulative and interactive effects of PCP toxicity under conditions of elevated temperatures or reduced dissolved oxygen.

Alternative Chronic Criteria**:** In the EPA's consultant review of the draft biological opinion (Chapman 1998), the reviewer proposed several different alternative chronic criteria. One proposal was to use acute toxicity values for carp (Verma *et al*. 1981, Hashimoto *et al*. 1982, and Matida *et al*. 1970). The study by Verma *et al*. (1981) on 3-day old carp larvae (*Cyprinus carpio*) found a 96-hour TL50 of 9.5 µg/L PCP, and a maximum acceptable threshold concentration (MATC) of between 0.5 to 0.6 µg/L PCP (based on survival and growth after 60 day exposure). However, the PCP in the test was not measured, nor was the pH. Because of the uncertainty in the pH and PCP concentration, we disagree that this study demonstrates that carp are more sensitive than salmonids to the acute effects of PCP. This study does however suggest that growth and mortality after chronic exposures is a sensitive endpoint for fish, given the low MATC derived. A study by Matida *et al*. (1971) further calls into question the contention by Chapman (1998) that carp are more sensitive than trout to PCP. In this study, both trout and carp fry were exposed to technical grade PCP under both acute and chronic exposures. The results of the acute study indicated that the 96-hour LC_{50} for trout are almost a factor of 3 lower than for carp. The differences in sensitivity were even more pronounced in the chronic study evaluating growth and mortality over 28 days for the trout, and 70 days for the carp. At 20 µg/L PCP, growth and mortality of carp fry were similar to that of the control after 70 days. In contrast, 20 µg/L PCP exposure to trout fry for only 28 days resulted in greater mortality than in the controls (13.3% vs. 3.3%), and decreased weight gain (39.&% versus 98.3%). At 8 and 3 µg/L PCP, mortality also appeared elevated compared to controls, and 8 µg/L appeared to affect growth. Use of this study to set criteria is problematic because the study design did not allow for evaluating the statistical significance and it does not appear that pH was measured during the test. Finally, the study by Hashimoto *et al*. (1982) using early life stage carp to test the acute toxicity of a commercial emulsifiable concentrate of PCP found little difference in sensitivity between the early life stage tested. This is in contrast to the findings of Van Leeuwen *et al*. (1985) who found sensitivity of salmon early life stage varied over 160-fold. In summary, the Services are unconvinced that using the carp studies to revise the final acute value and then derive a chronic criterion, as suggested by Chapman (1998), would be protective of early life stage salmonids**.**

Dr. Chapman (1998) also proposed revising the chronic criterion by using the existing final acute value of 10.56 µg/L PCP (at pH=6.5), along with two different revised acute-to-chronic ratios, to yield values of 2.02 µg/L and 2.94 µg/L (at pH = 6.5). This compares to an EPA proposed criterion of 4.04 µg/L (at pH = 6.5)**.** Such an approach may protect early life stage salmonids from significant mortality, although it is unclear if the greater toxicity of commercial PCPs, as compared to purified PCP, is accounted for in the final acute value. This approach would not be protective of sublethal effects on early life stage salmonids. Alternatively, Dr. Chapman proposes that the chronic criterion be 5.8 µg/L (at pH=7.4), based upon the highest concentration showing no adverse effect on mortality or growth (Chapman and Dominquez 1984). However, this study was conducted on purified PCP, and therefore it is not clear that this alternative criterion would

be protective of early life stage salmonids exposed to commercial forms of PCP. The study by Little *et al.* (1990), finding behavioral effects at 2 µg/L after only 4 days exposure and no effect at 0.2 µg/L of commercial PCP, suggests that a chronic criterion protective of both lethal and sublethal effects would be in the range of 0.2 to 2.0 µg/L (at pH=7.8). This range for the chronic criterion is also supported by the studies of Webb and Brett (1973) which found the threshold for effects on growth rate and food conversion efficiency to be around 2 µg/L (at pH=6.8).

The essential difficulty in devising an appropriate chronic criterion for protection of endangered salmonids is due to the apparent dearth of chronic toxicity tests which meet the EPA's exacting guidelines. The EPA has defaulted to using the approach of altering the final acute value by an acute-to-chronic ratio. It is clear from the numerous studies previously cited that sublethal effects on growth and behavior are the most sensitive endpoints for chronic exposure of PCP to salmonids, and that the approach of deriving a chronic criterion by adjusting the final acute value is inadequate. Therefore, there is an apparent need for EPA to conduct critical life-cycle, tests on salmonids in a manner which meets their requirements for deriving a chronic value, using commercial preparations of PCP. Such tests should include the effects of pH, elevated temperatures, and low dissolved oxygen on lethal and sublethal effects to salmonids, and should include sensitive endpoints such as growth and behavior. In the interim, the Services conclude that the existing data support a chronic criterion of between 0.2 to 2.0 µg/L PCP to be protective of early life stage salmonids (at pH 7.8).

Non-salmonid fish

There is limited information available on the acute toxicity of PCP to other federally listed fish species such as the Delta smelt, Lost River sucker, Modoc sucker, shortnose sucker, tidewater goby, unarmored three-spine stickleback, and Sacramento splittail. A study by Hedtke *et al.* (1986) determined a 96-hour LC_{50} of 85 µg/L for the white sucker (*Catastomus commersoni*) at a pH range of 7.4 to 8.4. The life stage or age of the fish was not provided. The sucker was more sensitive than the other two fish species tested, the fathead minnow (96hr. LC_{50} s = 120-510), and the bluegill (96hr. LC_{50} s = 200 and 270). A study by Adema and Vink (1981) found both the 48 hour and the 7 day LC_{50} of 450 µg/L for adult saltwater goby (*Gobus minutus*) at pH of 8.

To evaluate the early life stage effects on growth and behavior seen in salmonids, it is useful to compare those studies to other studies using similar endpoints with non-salmonid fish. Data on chronic toxicity to early life stage fish are also available for the fathead minnow, largemouth bass, and guppy. In a study by Brown *et al.* (1985), juvenile guppies were exposed to PCP (form not specified) for 4 weeks and general behavior, predator efficiency, and predator-prey response were observed. No effect was observed at 100 µg/L PCP, while behaviors indicative of decreased response to predators were observed at 500 and 700 µg/L. The lowest observable adverse effect level (LOAEL) of 500 µg/L is approximately 50 percent of the 96-hour LC_{50} of 1020 µg/L. In contrast, for salmonids the LOAEL for swimming activity of 2 µg/L is approximately 4 percent of the 96-hour LC_{50} value of 53 µg/L (Little *et al.* 1990). In a study on largemouth bass fry, Johansen *et al.* (1987) determined the chronic thresholds for food conversion efficiency and

growth to be both approximately 24 µg/L of reagent grade PCP. These chronic values are about 15 percent of the 96 hr. LC_{50} of 159 µg/L (Johansen *et al*. 1985). In a related study larval largemouth bass were exposed to reagent grade PCP for 8 weeks. The LOAEL for reduced feeding and growth was 45 µg/L, or approximately 16 percent of the 96-hour LC_{50} of 281 µg/L (Johansen *et al*. 1985; Brown *et al*. 1987). In a study on fathead minnows, embryos were exposed to PCP (93.7 percent pure) for 32 days and hatchability, weight, and survival were observed. No effects on hatchability or weight were seen at concentrations ranging from 16.9 to 176 µg/L. However, none of the early life stage minnows survived in the 176 µg/L test concentration, which is about 37 percent of the 96-hour LC_{50} determined for the egg. It appears, therefore, that chronic effects observed in early life stage salmonids occur at lower concentrations relative to the LC_{50} in other fish species tested. This is stated with caution however, because some of the chronic early life stage tests on non-salmonid fish were done with purified forms of PCP, which have been shown to be less toxic. For example, a 90 day study of early life stage fathead minnows conducted by Cleveland *et al*. (1982) using a composite commercial PCP determined a LOAEL for growth of 13 µg/L at pH=7.4, which is near the level of the proposed chronic criterion of 10 µg/L at that pH. Therefore, the limited literature on early life stage non-salmonid fish suggest that criteria which are protective of salmonids are likely to be protective of non-salmonids.

Bioaccumulative Effects

The proposed criteria for PCP use a BCF from water to fish tissue of 11. Eisler (1989) cites several studies showing much greater BCFs in fish. At 25 µg/L PCP, the BCF for trout muscle was 40 (as cited by Eisler). In studies cited in USEPA (1986b; Table 5) using non-salmonid fresh and saltwater fish, BCFs ranged from 7.3 to over 1000. It appears from the summary table in USEPA (1986b) that the BCF may be inversely related to the water concentration, with higher BCFs occurring at lower water concentrations of PCP. Chapman (1998) notes that a perusal of this same summary table suggests that BCFs seem to increase with decreasing pH. This phenomenon was demonstrated in goldfish exposed for 5 hours to PCP (Kishino and Kobayashi 1995). In that study, a BCF for PCP of 584 was determined at pH = 6; a BCF of 118 was found at pH=8; and a BCF of 8.9 was reported at pH=10. The duration of exposure may also determine the BCF; longer exposure durations may result in higher BCFs.

A study conducted by Niimi and McFadden (1982) found that PCP uptake from water is an important pathway for accumulation in fish over 115 days exposure. Water concentrations were less than 1 µg/L PCP, or well below the proposed water quality criteria. In their protocol, concentrations in fish were determined by removing intestinal content and discarding liver and gall bladder. BCFs in the study were in the range of 200 to 240, which are about 20-fold greater than the BCF used in the proposed water quality criteria.

The EPA consultant who reviewed the Services' draft biological opinion concurred, stating "Certainly the BCF of 11 does not appear to be appropriate based upon the information currently available" (Chapman 1998). Chapman notes that the Final Residue Value (FRV) approach was not used in the Great Lakes Initiative (USEPA 1995b), nor is a FRV identified in this proposed

rule or by the Services. While true, the choice of BCF should be based upon a more thorough review of the literature. Moreover, the higher BCF for PCP suggests that wildlife ingesting contaminated food may be at risk. Therefore, there is an apparent need for EPA to reevaluate the BCF, and to evaluate the effect of PCP on wildlife that ingest aquatic organisms exposed to PCP.

It has been established that commercial PCPs are significantly more toxic to aquatic organisms than are the purified forms of PCP (Eisler 1989). Also of concern is that impurities occurring in commercial preparations of PCP have been found to contain relatively high concentrations of polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), hexachlorobenzene, chlorinated phenoxyphenols, and chlorinated diphenyloxides. Chlorinated phenoxyphenols and other compounds found in PCP can be precursors to the formation of PCDD/Fs (Cleveland *et al*. 1982; Hamilton *et al*. 1986). PCDD/Fs are known to bioaccumulate in the environment and are also highly toxic to avian and mammalian wildlife. The bioaccumulation and chronic toxicity to wildlife of the other impurities found in commercial PCPs are not addressed by the proposed criteria. Therefore, there is an apparent need for EPA to also evaluate bioaccumulation and chronic toxicity to wildlife of the other impurities found in commercial PCPs.

Summary of Pentachlorophenol Effects on Listed Species

Based on the documented toxicity of pentachlorophenol to early life stage salmonids, with adverse effects seen at water concentrations between 2.5 to 7.5 times below the proposed chronic criterion, together with the potential for exposure of anadromous salmonids to occur, the Services conclude that the proposed numeric criteria are likely to significantly impair the survival and recovery of all listed anadromous salmonids, and are likely to adversely affect populations of the Lahontan cutthroat trout, Paiute cutthroat trout, and Little Kern golden trout if an exposure pathway is created within the habitat for these species.

The toxicity of PCP to non-salmonids, particularly the chronic toxicity, is difficult to assess due to a paucity of testing with the more toxic commercial grade PCP. In one of the few studies to use commercial PCP with non-salmonids the LOAEL for fathead minnow was within a few µg/L of the proposed chronic criterion for PCP. The Services therefore believe that chronic exposures at concentrations approaching the chronic criterion may also pose a potential hazard to some non-salmonid species. Among the non-salmonids, suckers and minnows appear more sensitive. The chronic criterion for PCP also fails to consider highly variable bioconcentration factors, an appropriate acute to chronic ratio, and differences in toxicity between commercial and purified PCP with regard to the acute to chronic ratio. The Sacramento splittail, delta smelt, Modoc sucker, shortnose sucker, and Lost River sucker all reside within watersheds in which pentachlorophenol exposure could occur. The Services therefore conclude that chronic exposure to PCP at concentrations below the criteria concentrations could have the potential to produce toxic effects in these species.

EPA Modifications Addressing the Services' April 9, 1999 draft Reasonable and Prudent Alternatives for Pentachlorophenol (PCP):

The above effect analysis considers the draft CTR as originally proposed in August of 1997. EPA has agreed by letter dated December 16, 1999, to modify its action for PCP per the following to avoid jeopardizing listed species.

A. *"By March of 2001, EPA will review, and if necessary, revise its recommended 304(a) chronic aquatic life criterion for PCP sufficient to protect federally listed species and/or their critical habitats. In reviewing this criterion, EPA will generate new information on chronic sub-lethal toxicity of commercial grade PCP, and the interaction of temperature and dissolved oxygen, to protect early life-stage salmonids. If EPA revises its recommended 304(a) criterion, EPA will then propose the revised PCP criterion in California by March 2002. If the proposed criterion is less protective than proposed by the Services in their opinion or if EPA determines that a proposed criterion is not necessary, EPA will provide the Services with a biological evaluation/assessment by March 2002 and will reinitiate consultation. EPA will keep the Services informed regarding the status of EPA's review of the criterion and any draft biological evaluation/assessment associated with the review. If EPA proposes a revised PCP criterion by March 2002, EPA will promulgate a final criterion as soon as possible, but no later than 18 months, after proposal."*

B. *"EPA will continue to use existing NPDES permit information to identify water bodies which contain permitted PCP discharges and Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and Resource Conservation and Reclamation Act (RCRA) sites that potentially contribute PCP to surface waters. EPA, in cooperation with the Services, will review these discharges and associated monitoring data and permit limits, to determine the potential for the discharge to impact federally listed species and/or critical habitats. If discharges are identified that have the potential to adversely affect federally listed species and/or critical habitat, EPA will work with the Services and the State of California to address the potential effects to the species. EPA will give priority to review data for fresh water bodies within the range of federally listed salmonids that currently lack a MUN designation as specified in the Regional Water Quality Control Boards' Basin Plans."*

Services' Assumptions regarding EPA modifications to the proposed action for removing jeopardy for PCP.

The Services anticipate the 304(a) criteria guidance for PCP will be revised by EPA to be sufficiently protective of salmonids by March 2001 and that criteria will be applied to all the appropriate water bodies within California no later than September 2003.

The Services recognize there are some scientific uncertainties and additional research is needed to determine the appropriate PCP criteria revision. Therefore, while EPA proposes to revise the criteria after generating new data, the Services assume that if new criteria are not developed, the

new information generated regarding the toxicity of commercial grade PCP and the interaction of temperature, pH and DO on sublethal acute and chronic toxicity to early life stage salmonids would conclusively demonstrate that the criteria as originally proposed by EPA (in the draft CTR) are sufficiently protective. The Services assume this information will be provided in sufficient detail to the Services in a biological assessment/evaluation to complete consultation by on the PCP criteria by March 2002, if necessary.

The Services assume a review of PCP monitoring and discharge data on existing hazards to salmonids in California water bodies will occur sometime during the year 2000 and that EPA will use existing authorities to identify and reduce PCP hazards to listed salmonids.

Cadmium

Adequacy of Proposed Chronic Criterion for Cadmium

The Services find that the chronic aquatic life criterion for cadmium proposed in the CTR does not protect listed salmonid and stickleback fish. The adequacy of cadmium criteria to protect certain sensitive species of aquatic organisms has apparently been in doubt for quite some time. In Eisler's (1985a) synoptic review of cadmium hazards, the author commented on the then current EPA 1980 cadmium criterion of 0.012 µg/L saying "even these comparatively rigorous criteria are not sufficient to protect the most sensitive species of freshwater insects, plants, crustaceans, and teleosts". (note to the reader: all cadmium concentrations discussed in this section are at 50 mg/L hardness unless noted otherwise). The EPA in their 1985 criteria document for cadmium (USEPA 1985b) raised the chronic criterion to 0.66 µg/L and noted that "if brook trout, brown trout, and striped bass are as sensitive as some data indicate, they might not be protected by this criterion". The 1985 criterion was also three to five times higher than the species mean chronic values for two cladoceran species which are important food sources for numerous juvenile and adult fish species. In 1995, the EPA again updated and increased the chronic cadmium criterion to 1.4 µg/L (USEPA 1996b) but did not make note of their own concerns that the previous criterion may not have been protective. In a ten year period the chronic cadmium criterion was increased 100-fold although there was doubt that certain salmonid species would be protected even with the lowest criterion. Pascoe and Mattey (1977) found in long-term tests that cadmium caused death in stickleback at concentrations measured at 0.8 µg/L (hardness of 103-111 mg/L as $CaCO_3$)and presumably causes toxic sub lethal effects at lower concentrations. Additional concerns of the Services over formulaic modifications of cadmium regulation on a dissolved basis are included in the formula-based metals section of this opinion.

Cadmium Criteria History

The EPA, in the 1976 criteria document, noted the sensitivity of salmonids and cladocerans (USEPA 1976). For soft water (0 - 75 mg/L), EPA recommended a 0.4 µg/L criterion specifically for salmonids and cladocerans. This was an order of magnitude below the recommended criterion for other nonsensitive species. The 1980 acute criterion was 1.2 µg/L and the chronic criterion was 0.012 µg/L using a hardness dependent formulas. Eisler did not consider these criteria

sufficiently protective of the most sensitive aquatic species (Eisler, 1985a).

In the document "Ambient Water Quality Criteria for Cadmium - 1984" (USEPA 1985b), the EPA had difficulties in determining final acute and chronic values. The acute data ranged widely with a salmonid being 3,400 times more sensitive than goldfish. When the final acute value was calculated, the value (8.917 µg/L) was higher than the acute toxicity to several trout species. To protect these commercially and recreationally important species, EPA lowered the value to 3.589 µg/L. This value was then divided by two for the acute aquatic criterion of 1.8 µg/L.

If sufficient data on chronic toxicity are available, the chronic criterion can be calculated using the same method as that used to develop the acute criterion or the chronic criterion can be determined by dividing the final acute value by the final acute to chronic ratio(ACR). In most cases for metals the EPA has used the ACR method (see USEPA metal criteria documents). The ACR is an acute effects concentration divided by a chronic effects concentration for the same species. However, the thirteen cadmium ACRs ranged from 0.9 to 433.8 and did "not seem to follow a pattern"(i.e. did not increase or decrease as the acute values increased or decreased, were not within a factor of ten). Based on the data, EPA decided that it was not "reasonable" to use a final ACR to determine a final chronic value. As an alternative, EPA took the thirteen genus mean chronic values and used the final acute value procedure to calculate a final chronic value. The chronic value initially calculated was 0.0405 µg/L. Although this value is over three times higher than the 1980 criterion of 0.012 µg/L it is still three to four times lower than the chronic toxicity concentrations for the most sensitive species tested. EPA then stated "however, because the thirteen genus mean chronic values contain values for five of the six freshwater genera that are acutely most sensitive to cadmium, it seemed more appropriate to calculate the final chronic value using N = 44, rather than N = 13...". N is the number of data points available and is used in one of the formulas to calculate the final acute or chronic values. In this case EPA used the acute N value (number of acute data points) to calculate the chronic value. It is not clear to the Services why using the acute N value to calculate the chronic criterion is "more appropriate". After making these adjustments a final chronic criterion of 0.66 µg/L was calculated. This value is higher than the chronic toxicity values for two cladocerans (see discussion below), is 16.5 times higher than the value calculated using the chronic N value, and is 55 times higher than the previous chronic criterion.

In 1995, EPA updated criteria for several pollutants including cadmium (USEPA 1996b). While some new acute data on cadmium were included and some older data were eliminated, it is unclear to the Services why a 1995 update did not use post 1986 cadmium references. The result of this recalculation was an acute value of 2.1 µg/L, a slight increase over the older value of 1.8 µg/L. For the chronic value, three of the old data points were eliminated because two values were determined using river water and in the other the cadmium concentration had not been directly measured. Two of the eliminated data points were for the second and fourth most sensitive genera. This had a significant effect on the calculations since the data for the four most sensitive genera are ultimately used in the final chronic value calculation. Three new data points were added to the original 1985 chronic data set. One became the highest chronic value in the data set

at 20 µg/L for an oligochaete (an "aquatic earthworm") but this value does not directly affect the calculations. The other two new values were eventually not used in the calculations because data for a more sensitive species in that genera was used as the genus mean chronic value for the final calculations. The update again used the acute N value to calculate the chronic criterion although the elimination of data made the EPA's reason for using the acute N value rather than the chronic N value less "appropriate" because the twelve genus mean chronic values now contain values for four (rather than five) of the six freshwater genera that are acutely most sensitive to cadmium. The 1995 recalculation doubled the chronic value to 1.4 µg/L from the old 0.66 µg/L and is over 100 times higher than the 0.012 µg/L criterion of 1980. If EPA had used the chronic N value to calculate the chronic criterion a value of 0.096 µg/L would have been obtained.

As previously noted, EPA did not use the ACR method to determine the chronic criterion because the ratios did not follow any clear trends. If the ACR method had been used there are several options that can be considered: 1) use all fresh and salt water ACRs available, 2) use all fresh water ACRs, or 3) use the fresh water ACRs of those species with mean acute values closest to the final acute value. Taking the 1985 data as updated in 1995 the ACR chronic values would be 1) 0.11 µg/L, 2) 0.07 µg/L, and 3) 0.18 µg/L. For the third method, three ACR values were used and included the two most chronicly sensitive species (daphnia and chinook salmon) which were also two of the four most acutely sensitive species. Also, the three species mean acute values were within a factor of ten.

Based on the evaluations above using the chronic N value and looking at several ACR methods, it appears that a continuous concentration criterion for cadmium that would be protective of salmonids and stickleback is somewhere between 0.096 and 0.180 µg/L, but probably would still not protect cladocerans.

Considering that the 1985 criteria document noted that the chronic criterion may not be protective of some cladoceran and trout species, it appears unusual that the 1995 update, which doubles the chronic criterion, makes no mention of this lack of protection. Since the original 1985 chronic cadmium criterion may not have been protective of cladocerans and several trout species, the Services conclude the 1995 updated chronic criterion will not be protective of listed salmonid species either and therefore the proposed CTR chronic criterion for cadmium will not be protective.

Considering that the only data available on cadmium toxicity to threespine stickleback shows that the species is highly sensitive at concentrations below the proposed criterion, the Services conclude that the proposed chronic criterion will not be protective of this species.

The Services also conclude that the additional loss of protection due to the proposed regulation of cadmium on a dissolved basis using a formula-based criterion, as discussed elsewhere in this opinion, adds to the likelihood of adverse effects to listed salmonid species and the unarmored threespine stickleback.

Cadmium Hazards to Aquatic Organisms

Sources

Eisler's synoptic review (1985a), EPA's criteria document (USEPA 1985b), Sorensen (1991), and Moore and Ramamoorthy (1985) provide a good summary of cadmium sources and pathways. Cadmium is not a biologically essential metal. It is a soft metal with properties similar to zinc. Cadmium is most often found with sulfide ores and is frequently associated with other metals such as zinc, copper, and lead. Mining and ore smelting are significant sources of cadmium to the environment via direct discharge of mine drainage and atmospheric deposition. Cadmium is frequently associated with industrial discharges and stormwater runoff. Uses of cadmium include electroplating, pigments, plastic stabilizers, batteries, and electronic components. Background concentrations of cadmium in freshwater ranges from <0.01 to 0.2 µg/L and are usually less than 0.05 µg/L in waters unimpacted by man (USEPA 1985b, Eisler 1985a, Wren et al., 1995). The maximum background concentrations are close to or at concentrations that can be harmful to sensitive aquatic species. Human activities can raise cadmium concentrations to levels >1 µg/L.

Pathways

For cadmium and other dissolved metals the most direct pathway to aquatic organisms is via the gills. Cadmium is also directly taken up by bacteria, algae, plants, and planktonic and benthic invertebrates. Another biologically significant pathway for exposures of aquatic organisms to cadmium is through consumption of contaminated aquatic detritus, plants, invertebrates, and other food items. Dietary exposure and association with sediment is significant in cadmium accumulation in fish species (Sorensen 1991). Omnivorous fish tend to accumulate higher levels of cadmium than carnivorous fish and bottom feeding fish tend to accumulate more cadmium than free-swimming fish feeding in the water column.

General Toxicity of Cadmium

Cadmium damages gill, liver, kidney, and reproductive tissue (Eisler 1985a; Sorensen 1991; Moore and Ramamoorthy 1984). Acute mechanisms of cadmium toxicity to fish do not appear to be the same as chronic mechanisms. In acute tests cadmium accumulates in gill tissue to a greater extent than elsewhere, whereas, in chronic tests at lower concentrations, cadmium accumulates more in liver and kidney tissue. The principle acute effect is gill toxicity leading to an aquatic organism's inability to breath. Long term effects include the inability to regulate plasma constituents, produce healthy bones, and reproduce. Cadmium will compete with essential metals such as zinc for enzyme binding sites, thus disrupting normal enzyme functions. Hypocalcemia also occurs due to exposure to cadmium thus causing muscular and neural abnormalities. Cadmium is considered a teratogenic substance.

The toxicity of cadmium varies greatly among aquatic species (USEPA 1985b). Mean acute values for sensitive life stages of freshwater fish range from 1.6 µg/L for brown trout to 7,685

µg/L for mosquitofish. The most sensitive species being salmonids, striped bass, and cladocerans. Acute toxicity for chinook salmon is 4.254 µg/L. Mean acute values for less sensitive species range as high as 1,200 µg/L for midge larvae to 12,755 µg/L for crayfish. The goldfish mean acute value is 8,325 µg/L. Hardness, pH, alkalinity, salinity, and temperature can significantly affect cadmium toxicity.

USEPA (1985b) shows mean chronic toxicity concentrations for two cladocerans at 0.1918 µg/L and 0.1354 µg/L. USEPA (1996b), noted additional low chronic values for cladocerans at 0.12, 1.25, 3.919, 4.0, and 6.096 µg/L. Four of the cladoceran values were not used in the calculation of the 1995 criterion for reasons noted above. As sensitive as cladocerans seem to be it is possible that the life stage of cladocerans being used in most bioassays are not the most sensitive. Shurin and Dodson (1997) found that sexual reproduction in cladocerans is more sensitive to toxicants than the asexual reproductive stage and that most bioassays utilize daphnia during the asexual phase because they are well fed and cultured under low stress situations. Under stress (low temperature, drought, low food supply) cladocerans and other zooplankton use sexual reproduction to produce resting eggs that can remain dormant for months to years until more favorable conditions return. The loss or a decrease in the production of resting eggs can have a significant long-term effect on the populations these species. Snell and Carmona (1995) found that for a rotifer zooplankton, sexual reproduction was more strongly affected by several toxicants, including cadmium, than asexual reproduction. The authors concluded that the "level of toxicants presently allowable in surface waters…may expose zooplankton populations to greater ecological risks than is currently believed."

Mean chronic values in fish range from 2.362 µg/L for the brook trout to 16.32 µg/L for bluegill while the mean chronic value for early life stage chinook salmon is 2.7 µg/L. Pascoe and Mattey (1977) found that cadmium at concentrations as low as 1 µg/L can be toxic to the three-spined stickleback after 33 days. Acute to chronic ratios also vary greatly among test organisms and range from 0.9 to 433.8.

There is very little information on the toxicity of cadmium to amphibians. USEPA (1985b) notes data on three species. The EC_{50} (death and deformity) of embryo and larval narrow-mouthed toads (*Gastrophyryne carollnensis*) after seven days at a hardness of 195 mg/L was 40 µg/L. The 48 hr LC_{50} (death) of African clawed frogs (*Xenopus laevis*) at 209 and 170 mg/L hardness was 11,700 and 3,200 µg/L respectively. After 100 days African clawed frogs showed signs of inhibited development at 650 µg/L at a hardness of 170 mg/L. Finally, marbled salamander (*Ambystoma opacum*) embryos and larvae had an EC_{50} (death and deformity) of 150 µg/L at a hardness of 99 mg/L after eight days. The sensitive life stages of these species appear to be similar in their sensitivity to cadmium as adult goldfish and fathead minnows. Concentrations of cadmium that would be protective of salmonids would protect amphibians.

Summary of Cadmium Criteria Effects to Listed Species

Fish

Salmonid species are particularly sensitive to cadmium. USEPA (1996c) shows mean acute toxicity values of sensitive life stages for coho salmon at 5.894 µg/L, chinook salmon at 4.254 µg/L, rainbow trout at 3.589 µg/L, and brown trout at 1.638 µg/L. Chronic values for coho salmon, chinook salmon, brown trout, and brook trout are 2.324 µg/L, 2.694 µg/L, 7.372 µg/L, and 2.194 µg/L respectively. These low concentrations reduce growth, survival, and fecundity.

Increased water temperature increases cadmium toxicity (Eisler 1985a; USEPA 1985b; Sorensen 1991; and Moore and Ramamoorthy 1985). Increased temperature is a major problem for listed salmonids in California due, in part, to logging activities decreasing riparian shading of streams and dams increasing water temperatures in reservoirs.

Cladocerans and other invertebrates are very sensitive to cadmium. They also provide significant food sources for early life stage salmonids and other aquatic organisms that are themselves prey items for salmonids. It also appears that the least sensitive reproductive stage of zooplankton such as cladocerans is more often used for bioassays leading to an underestimate of their sensitivity to various toxicants including cadmium (Shurin and Dodson 1997, Snell and Carmona 1995). A loss of this prey base can indirectly impact salmonids and stickleback.

Pascoe and Cram (1977) found lethal chronic toxicity of cadmium to the three-spined stickleback (*Gaserosteus aculeatus L.*) at all tested concentrations with the lowest concentration tested being 300 µg/L. An interaction was also found between the incidence of parasitism and sensitivity to cadmium. Subsequently Pascoe and Mattey (1977) performed a long-term (89 day) study on three-spined stickleback at concentrations of cadmium from 100,000 µg/L to 1 µg/L. Lethality to the stickleback was again found at all concentrations tested. The authors determined a 96 h LC_{50} of 23,000 µg/L but went on to say; "The results confirm earlier work (Pascoe & Cram 1977) that cadmium is highly toxic to sticklebacks. It is now seen to cause death at concentrations as low as 0.001 mg l^{-1} [1 µg/L] in water of total hardness 103-111 mg l^{-1} as $CaCO_3$ at 15° C, and presumably causes toxic sub lethal effects at lower concentrations." The median period of survival at 1 µg/L was 48,000 minutes (33.3 days). At 3.2 µg/L the median survival time was 23,000 minutes (16 days). The nominal concentration at this low level was 0.001 mg l^{-1} while the measured concentration was 0.0008 mg l^{-1} (0.8 µg/L). This chronic data, while cited, was not used by EPA in criteria calculations. However, the Services and EPA must consider this relevant and available data for evaluation of potential effects of permissible cadmium concentrations to the listed subspecies of the stickleback *(G. aculeatus williamsonii).*

The Services believe that all ESUs and runs of coho and chinook salmon and steelhead trout, Lahontan cutthroat trout, Paiute cutthroat trout, Little Kern golden trout, along with the unarmored threespine stickleback are likely to be adversely affected by concentrations of cadmium at or below those that would be allowed in the proposed CTR.

EPA modifications addressing the Services' April 9, 1999 draft Reasonable and Prudent Alternatives for Cadmium:

The above effect analysis evaluates the draft CTR as originally proposed in August of 1997. EPA has agreed by letter dated December 16, 1999, to modify its action for cadmium per the following to avoid jeopardizing listed species.

"EPA will develop a revision to its recommended 304 (a) chronic aquatic life criterion for cadmium by January 2001 to ensure the protection of federally listed species and/or critical habitats and will propose the revised criterion in California by January 2002. However, if EPA utilizes the revised metals criteria model referred to below, EPA will develop a revision to its recommended 304(a) criterion by January 2002 and will propose the revised criterion in California by January 2003. EPA will solicit public comment on the proposed criteria as part of its rulemaking process, and will take into account all available information, including the information contained in the Services' opinion, to ensure that the revised criterion will adequately protect federally listed species. If the revised criterion is less stringent than that proposed by the Services in the opinion, EPA will provide the Services with a biological evaluation/assessment on the revised criterion by the time of the proposal to allow the Services to complete a biological opinion on the proposed cadmium criterion before promulgating final criteria. EPA will provide the Services with updates regarding the status of EPA's revision of the criterion and any draft biological evaluation/assessment associated with the revision. EPA will promulgate final criteria as soon as possible, but no later than 18 months, after proposal. EPA will continue to consult, under section 7 of ESA, with the Services on revisions to water quality standards contained in Basin Plans, submitted to EPA under CWA section 303, and affecting waters of California containing federally listed species and/or their habitats. EPA will annually submit to the Services a list of NPDES permits due for review to allow the Services to identify any potential for adverse effects on listed species and/or their habitats. EPA will coordinate with the Services on any permits that the Services identify as having potential for adverse effects on listed species and/or their habitat in accordance with procedures agreed to by the Agencies in the draft MOA published in the Federal Register at 64 F. R. 2755 (January 15, 1999) or any modifications to those procedures agreed to in a finalized MOA."

Services' Assumptions Regarding EPA's Modifications for Removing Jeopardy for Cadmium.

The Services assume the 304(a) cadmium chronic aquatic life criterion can and will be revised by EPA to be sufficiently protective of sticklebacks and salmonids in California by no later than January 2001. The Services assume that this revision will result in lowering the permissible concentrations of cadmium. Further, the Services assume this scientific guidance can and will be used in revising permits during the interim period prior to promulgation of this criterion in California.

If, however, the criterion proposed by EPA is less stringent than that suggested by the effects analysis of the Services, EPA will provide a new biological assessment with new information that indicates why a criterion less stringent than that suggested by the Services will be sufficiently protective.

The Services assume that because EPA offered to revise the chronic aquatic life criterion for cadmium by January 2001 that this is achievable by EPA. There is a discrepancy in EPA's letter about when a new criteria model for metals will be developed per paragraphs IV and V in EPA's December 16, 1999 letter. June of 2003 is presented as the date of the model revision for metals criteria, but paragraph IV states the 304a criterion for cadmium per the new model would be ready by January 2002. The Services' view is that an earlier revision as proposed by EPA without the new metals model that protects these listed species is preferable and should be pursued by EPA to provide the earliest possible increase in protection.

Metals

Adequacy of Proposed Criteria

Metals addressed in the CTR include: arsenic (As), cadmium (Cd), trivalent chromium (CrIII), hexavalent chromium (CrVI), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se) in saltwater, silver (Ag), and zinc (Zn). Although mercury, cadmium and selenium are discussed in separate sections of this biological opinion, this section on conversion factors and water effect ratios also applies to proposed mercury and saltwater selenium criteria. The formula-based metals are included in this single discussion as a group because the key issues of how dissolved metal criteria are derived and the implications are similar for each of them. That is, the formula-based metal method does not sufficiently consider the environmental fate, transport, and transformations of metals in natural environments.

Use of Formulas

The EPA proposes to promulgate within the CTR aquatic life criteria that are formula-based for the following metals: As, Cd, Cr(III), Cr(VI), Cu, Pb, Hg, Ni, Se (in saltwater), Ag, and Zn. To determine criteria for these metals that are applicable to a given water body, site-specific data must be obtained, input to a formula, and numeric criteria computed. There are three types of site-specific data that may be necessary to determine and/or modify the criterion for a metal at a site: water hardness, conversion factors and translators, and water effect ratios. The following is a brief description of these types of data.

1. Formulas for Cd, Cu, Cr(III), Pb, Ni, Ag, and Zn are water hardness dependent. The Services assume that the measure of hardness referred to in the CTR is a measure of the water hardness due to calcium and magnesium ions. By convention, hardness measurements are expressed in terms of the mg/L of $CaCO_3$ required to contribute that amount of calcium + magnesium hardness. Therefore, the site-specific hardness is determined at a site, expressed as mg/L of $CaCO_3$, then input to the criteria formulas for each metal. Originally criteria were determined using data on the total metal concentration (dissolved and particulate) in the test water. Thus, the general formula for a hardness based chronic criterion or Criterion Continuous Concentration (CCC) on a total metal basis is:

$$CCC = e^{(m[\ln(\text{hardness})]+b)}.$$

As an example, for Cu, the following data can be input to the general formula above: a site hardness of 40 mg/L and the slope (m) and intercept (b) for copper hardness dependent chronic toxicity (from CTR Table 2). The Criterion Continuos Concentration (CCC) for Cu, on a total basis would be:

$$CCC\ (\text{total}) = e^{(0.8545[\ln(40)]+(-1.702))}$$
$$= 4.3\ \mu g/L$$

Criteria for Cd, Cu, Cr(III), Pb, Ni, Ag, and Zn can not be found directly by seeking out a reference like the CTR, because numbers listed in such tables are usually based on the assumption that the site-specific hardness is 100 mg/L (the CCC for Cu at this hardness is 9.3 µg/L). Criteria for these metals require that site-specific hardness is measured and input to the formula, as demonstrated above.

2. Formulas for all the metals also include a total-to-dissolved conversion factor (CF) based on the fraction of the metal that was in a dissolved form during the laboratory toxicity tests used to develop the original total based criteria. Criteria as proposed in the CTR would be on a dissolved basis. Table 1 in the CTR lists the CFs for the metals. The modified formula becomes:

$$CCC\ (\text{dissolved}) = CF \times e^{(m[\ln(\text{hardness})]+b)}.$$

Using the hardness, slope, and intercept values from above and the CF from Table 1 in the CTR, the dissolved Cu chronic criterion would be:

$$CCC\ (\text{dissolved}) = 0.96 \times e^{(0.8545[\ln(40)]+(-1.702))}$$
$$= 4.1\ \mu g/L$$

There is an added level of complexity in the computations of criteria for Cd and Pb because the CFs for these metals are themselves hardness dependent. For example, the formula to derive the hardness-dependent CF for the chronic (CCC) Cd criterion is:

$$CF = 1.101672-[(\ln\{\text{hardness}\})(0.041838)]$$

This hardness-specific CF would then be entered into the formula for Cd and the criterion would be calculated similar to the example above.

If a total maximum daily load (TMDL) is needed to regulate discharges into an impaired water body, the dissolved criterion must be converted or translated back to a total value so that the TMDL calculations can be performed. The translator can simply be the CF (divide the dissolved criterion by the CF to get back to the total criterion) or site-specific data on total and dissolved

metal concentrations in the receiving water are collected and a dissolved-to-total ratio is used as the translator.

3. Formulas for all the metals listed above also include a Water Effects Ratio (WER), a number that acts as a multiplication factor. If no site-specific WER is determined, then the WER is presumed to be 1 and would not modify a formula result. A WER purportedly accounts for the difference in toxicity of a metal in a site water relative to the toxicity of the same metal in reconstituted laboratory water. The contention is that natural waters commonly contain constituents which "synthetic" or "reconstituted" laboratory waters lack, such as dissolved organic compounds, that may act to bind metals and reduce their bioavailability. Where such constituents act to modify the toxicity of a metal in a site water compared to the toxicity of the same metal in laboratory water, a "water effect" is observed.

Example WER calculation:

Suppose the LC_{50} of Cu in site water is 30 µg/L.
Suppose the LC_{50} of Cu in laboratory water is 20 µg/L.
As before assume a site hardness of 40 mg/L.
The freshwater conversion factor (CF) for Cu = 0.96.

$$\text{WER} = \frac{\text{Site } \mathbf{LC_{50}}}{\text{Lab } \mathbf{LC_{50}}} = \frac{30 \text{ µg/L}}{20 \text{ µg/L}} = 1.5$$

$$\text{Cu Site-Specific CCC} = \text{WER} \times \text{CF} \times e^{(m[\ln(40)]+b)}$$
$$= 1.5 \times 0.96 \times 4.3$$
$$= 6.2 \text{ µg/L}$$

What follows are discussions of the Services' concerns regarding the applications of WER, CF and the attendant translators, and deficiencies of the hardness-dependent factors in formula-based determinations of criteria for As, Cd, Cr (III), Cr (VI), Cu, Pb, Hg, Ni, Se (in saltwater), Ag, and Zn.

Water Effect Ratios

Except in waters that are extremely effluent-dominated, WERs are > 1 and result in higher numeric criteria. Note that, in the examples above, use of a site-specific WER for copper raised the criterion concentration allowed at the site from 4.1 µg/L to 6.2 µg/L, an increase of 50 percent. A WER may be more important than site water hardness or metal-specific conversion factors and translators in determining a criterion and hence the metal loading allowed (see

hardness and ading discussions below).

EPA has published guidelines for determining a site-specific WER, which outline procedures for water sampling, toxicity testing, acclimating test organisms, etc. (USEPA 1994). When site water toxicity is lower than laboratory water toxicity, criteria may be raised because: 1) differences in calcium to magnesium ratios in hardness between laboratory water and site water can significantly alter the WER; 2) toxicity testing for WER development is not required across the same range of test organisms used in criteria development; and 3) the inherent variabilities associated with living organisms used in toxicity testing can be magnified when used in a ratio.

EPA guidelines for WER determinations (USEPA 1994) instruct users to reconstitute laboratory waters according to protocols that result in a calcium-to-magnesium ratio of ~0.7 across the range of hardness values (USEPA 1989, 1991). This proportion (~0.7) of calcium to magnesium is far less than the ratio found in most natural waters (Welsh *et al*. 1997). The Services agree with Welsh *et al*. (1997) that imbalances in Ca-to-Mg ratios between site waters and dilution waters may result in WERs which are overestimated because calcium ions are more protective of metals toxicity than are magnesium ions. The EPA has noted this problem with determining WERs but limits the suggested correction of matching the laboratory Ca-to-Mg ratio and the site ratio to a single sentence at the end of the proposed rule. Thus, the significance and correction of this problem is not adequately addressed.

EPA metal criteria are based on over 900 records of laboratory toxicity tests (USEPA 1992) using hundreds of thousands of individual test organisms, including dozens of species across many genera, trophic levels, and sensitivities to provide protection to an estimated 95 percent of the genera most of the time (USEPA 1985f). The use of a ratio based WER determined with 2 or 3 test species limits the reliability of the resultant site-specific criteria and calls into question the level of protection provided for families or genera not represented in the WER testing

The inherent variability of toxicity testing can also have a significant effect on the final WER determination, especially because it is used in a ratio. As discussed above, the EPA has developed its criteria based on a relatively large database. However, even with such a large database variability in test results can still cause difficulty in determining a criteria value. For example, Cd data were so variable that EPA abandoned the acute to chronic ratio method of determining the chronic criterion (USEPA 1985b). Instead, EPA applied the acute method to derive a chronic value. The EPA criteria document for Cd (USEPA 1985b) notes a chronic value for chinook salmon of 1.563 µg/L with a range of 1.3 to 1.88 µg/L. This is a variability of 17 percent in either direction, which is rather good (inter and intra laboratory variability higher than 17 percent is not unusual). Therefore, if this data is used in a ratio such as a WER, the variability alone could result in a 34 percent difference in the values used. A potential WER using such data could range from 0.7 to 1.4. Thus, a site-specific criteria could increase by 40 percent due to natural variability in the toxicity testing alone. In development of a site-specific WER, fewer tests are conducted and with fewer species, increasing the likelihood that natural variation in toxicity test results could affect the outcome. Care should also be taken to make sure that test results

between lab and site water are significantly different. If 95 percent confidence intervals for the tests overlap then they are likely not significantly different and should not be used to determine a WER. Thus, toxicity tests should be conducted and carefully evaluated to minimize experimental variance when collecting data to calculate WERs.

Zooplankton such as cladocerans (*Daphnia sp.*) are commonly used in bioassays to determine national and site-specific criteria or develop WERs and translation factors. As sensitive as cladocerans seem to be it is possible that the life stage of cladocerans being used in most bioassays are not the most sensitive. Shurin and Dodson (1997) found that sexual reproduction in cladocerans is more sensitive to toxicants than the asexual reproductive stage and that most bioassays utilize daphnia during the asexual phase because they are well fed and cultured under low stress situations. Under stress (low temperature, drought, low food supply) cladocerans and other zooplankton use sexual reproduction to produce resting eggs that can remain dormant for months to years until more favorable conditions return. The loss or a decrease in the production of resting eggs can have a significant long-term effect on the populations of these species. Snell and Carmona (1995) found that for a rotifer zooplankton, sexual reproduction was more strongly affected by several toxicants, including cadmium, than asexual reproduction. The authors concluded that the "level of toxicants presently allowable in surface waters . . . may expose zooplankton populations to greater ecological risks than is currently believed." Other metals may also be more toxic to the sexual stage of zooplankton adding additional doubt to the protectiveness of some criteria and WERs.

Procedures for acclimation of test organisms prior to toxicity testing may also be inadequate to assure meaningful comparisons between site and laboratory waters. For the reasons stated above, the Services believe that the EPA procedures for determining WERs for metals may result in criteria that are not protective of threatened or endangered aquatic species. Thus, WERs of three (3) or less are unacceptable because they are likely within the variance of the toxicity tests. WERs over three must be carefully developed and evaluated to ensure that listed species will be protected.

Conversion Factors and Translators

EPA derived ambient metals criteria from aquatic toxicity tests that observed the dose-response relationships of test organisms under controlled (laboratory) conditions. In most of these studies, organism responses were plotted against nominal test concentrations of metals or concentrations determined on unfiltered samples. Thus, until recently metals criteria have been expressed in terms of total metal concentrations. Current EPA metals policy (USEPA 1993a) and the CTR in particular propose that criteria be expressed on a dissolved basis because particulate metals contribute less toxicity than dissolved forms. EPA formulas for computing criteria thus are adjusted via a conversion factor (CF), so that criteria based on total metal concentrations can be "converted" to a dissolved basis. Metals for which a conversion factor has been applied include arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver, and zinc.

The CF is a value that is used to estimate the ratio of dissolved metals to total recoverable metals to adjust the former criteria based on total metal to yield a dissolved metal criterion. A CF based on the premise that the dissolved fraction of the metals in water is the most bioavailable and therefore the most toxic (USEPA 1993a, 1997c). The presumption is that the dose/response relationships found in toxicity tests would be more precise if "dissolved" metal concentrations were determined in test solution samples that have been filtered to remove the larger-sized, particulate metal fraction. The term "total" metal refers to metal concentrations determined in unfiltered samples that have been acidified (pH < 2) before analysis. The term "dissolved" metal refers to metal concentrations determined in samples that have been filtered (generally a 0.45-micron pore size) prior to acidification and analysis. Although it is clear that concentrations determined in a procedurally-defined dissolved sample are not accurate measures of dissolved metals, it may be premature to recommend immediate changes to the current procedure (Chapman 1998). Particulate metals can be single atoms or metal complexes adsorbed to or incorporated into silt, clay, algae, detritus, plankton, etc., which can be removed from the test water by filtration through a 0.45 micron filter. A CF value is always less than 1 (except for As which is currently 1.0) and is multiplied by a total criterion to yield a (lower) dissolved criterion. For example, CF values for Cd, Cu, Pb, and Zn, are 0.944, 0.960, 0.791, and 0.978 respectively (USEPA 1997c). The CF values approach 100 percent for several metals because they are ratios determined in laboratory toxicity-test solutions, not in natural waters where relative contributions of waterborne particulate metals are much greater. The California Department of Fish and Game (CDFG 1997) has commented that particulate fractions in natural waters in California are often in the range of 80 percent, which would equate to a dissolved-to-total ratio of 0.2.

To convert metals criteria, EPA reviewed test data that reported both total and dissolved concentrations in their test waters and also conducted simulations of earlier experiments to determine the dissolved-to-total ratios (USEPA 1992, 1995a, 1997c). In this way, the historical toxicity database could be preserved and a large number of new toxicity tests would not have to be performed. Overall, the CFs proposed in the CTR are based upon roughly 10% of the historical database of toxicity tests. CF values for As and Ni were based on only 1 study each, comprising 11 records. CF values for Cr were based on only 2 studies, while the estimated CF for Pb was based on 3 studies, comprised of only 3 records. Although additional confirmatory studies were performed to develop the CFs, the database available appears to be limited and calls into question the defensibility of the CFs determined for these metals.

Ultimately the scientifically most defensible derivation of dissolved metals criteria should be based on reviews of new laboratory investigations because:

1. the several water quality variables that modulate metal toxicity may not have been properly controlled, measured, reported, or manipulated over ranges that are environmentally realistic and necessary to consider if site-specific criteria are to be proposed (see section on hardness);

2. it is likely that most toxicity tests measured organism responses in terms of traditional endpoints such as mortality, growth, reproductive output. These may not be sufficient for

determining the toxic effects of metals in test waters manipulated to reflect environmental (site) conditions (see section on hardness);

3. the test waters contained very low contributions from particulate metals to the total metal concentrations. These proportions are not environmentally realistic; and

4. the present EPA criteria for metals lack meaningful input and modification from metals toxicity research done in the last decade.

Points 1 and 2 above are discussed in this final biological opinion in the hardness section dealing with the use of water hardness as a general water quality "surrogate". Point 3 is illustrated by the fact that the CF's proposed in the CTR for several metals are near a value of 1.0. This indicates that the toxicity tests reviewed to derive dissolved-based criteria exposed test organisms in waters that contained very low concentrations of particulate metals. For example, the CF values for Cd, Cu, Pb, and Zn, are 0.944, 0.960, 0.791, and 0.978 respectively (USEPA 1997c), meaning that particulate metal percentages were (on average) 5.6%, 4.0%, 20.9%, and 2.2%. These percentages are much lower than found in many natural waters. The California Department of Fish and Game, in their comments to the EPA on the proposed CTR, has stated that particulate fractions in natural waters in California are often in the range of 80 percent (CDFG 1997), which would equate to a dissolved-to-total ratio of 0.2. It is clear that the historical toxicity database does not include studies of the toxic contributions of particulate metals under environmentally realistic conditions. Improved assessments are necessary to develop adequately protective, site-specific criteria.

The EPA Office of Water Policy and Technical Guidance has noted that particulate metals contribute some toxicity and that there is considerable debate in the scientific community on this point (USEPA 1993a). While the Services agree that dissolved metal forms are generally more toxic, this is not equivalent to saying that particulate metals are non-toxic, do not contribute to organism exposure, or do not require criteria guidance by the EPA. Few studies have carefully manipulated particulate concentrations along with other water constituents, to determine their role(s) in modulating metals toxicity. Erickson *et al.* (1996) performed such a study while measuring growth and survival endpoints in fish and suggested that copper adsorbed to particulates cannot be considered to be strictly non-toxic. Playle (1997) cautions that it is premature to dismiss particulate-associated metals as biologically unavailable and recommends the expansion of fish gill-metal interaction models to include these forms. The Service is particularly concerned that investigations have not been performed with test waters that contain both high particulate metal concentrations and dissolved concentrations near the CTR-proposed criteria concentrations. Despite a paucity of information about the aquatic toxicity of particulate metals, the CTR proposes that compliance would be based on removing (filtering) these contaminants from a sample prior to analysis. It would be prudent to first conduct short-term and longer term studies, as well as tests that expose organisms other than fish.

Particulates may act as a sink for metals, but they may also act as a source. Through chemical,

physical, and biological activity these metals can become bioavailable (Moore and Ramamoorthy 1984). Particulate and dissolved metals end up in sediments but are not rendered entirely nontoxic nor completely immobile, thus they still may contribute to the toxicity of the metal in natural waters.

Particulate metals have been removed from the regulatory "equation" through at least two methods: the use of a CF to determine the dissolved metal criteria, and the use of a translator to convert back to a total metal concentration for use in waste load limit calculations. When waste discharge limits are to be developed and TMDLs are determined for a receiving waterbed, the dissolved criterion must be "translated" back to a total concentration because TMDLs will continue to be based on total metals.

EPA provides three methods in which the translation of dissolved criteria to field measurements of total metal may be implemented. These three methods may potentially result in greatly different outcomes relative to particulate metal loading. These methods are:

1. Determination of a site specific translator by measuring site specific ratios of dissolved metal to total metal and then dividing the dissolved criterion by this translator. As an example: a site specific ratio of 0.4 (40% of the metal in the site water is dissolved) would result in a 2.5 fold increase in the discharge of total metal. The higher the fraction of particulate metal in the site water the greater the allowable discharge of total metal. See the discussion and Table 9 below. This is EPA's preferred method.

2. Theoretical partitioning relationship. This method is based on a partitioning coefficient determined empirically for each metal and when available the concentration of total suspended solids in the site specific receiving water.

3. The translator for a metal is assumed to be equivalent to the criteria guidance conversion factor for that metal (use the same value to convert from total to dissolved and back again).

Since translators are needed to calculate discharge limits they become important in determining the total metals allowed to be discharged (see also loading discussion for individual metals below. In the economic analysis performed by the EPA and evaluated by the State Board (SWRCB 1997), it was estimated that translators based on site-specific data will decrease dischargers costs of implementing the new CTR criteria by 50 percent. This cost savings is "directly related to the less stringent effluent limitations that result from the use of site-specific translators." This implies a strong economic incentive for dischargers to reduce costs by developing site-specific translators and ultimately being allowed to discharge more total metals. This conclusion regarding the impact of site specific translators is supported by documents received from EPA (USEPA 1997d). EPA performed a sensitivity analysis on the effect of the site specific translator, which relies on determining the ratio of metal in water after filtration to metal in water before filtration in downstream waters. EPA's analysis indicated that use of a site-specific translators to calculate criteria would result in greater releases of toxic-weighted metals loads above the option where the

Cfs are used as the translators. The potential difference was estimated to be between 0.4 million and 2.24 million "toxic weighted" pounds of metals discharged to California waterways.

The Services believe that the current use of conversion factors and site specific translators in formula-based metal criteria are not sufficiently protective of threatened and endangered aquatic species because:

1. particulate metals have been removed from the regulatory equation even though chemical, physical, and biological activity can subsequently cause these particulate metals to become bioavailable;

2. the criteria are developed using toxicity tests that expose test organisms to metal concentrations with very low contributions from particulate metals;

3. toxicity tests do not assess whether the toxic contributions of particulate metals are negligible when particulate concentrations are great and dissolved concentrations are at or near criteria levels;

4. this method has the potential to significantly increase the discharge of total metal loads into the environment even though dissolved metal criteria are being met by a discharger; and

5. the premise ignores the fact that water is more than a chemical medium, it also physically delivers metals to the sediments.

Hardness

The CTR should more clearly identify what is actually to be measured in a site water to determine a site-specific hardness value. Is the measure of hardness referred to in the CTR equations a measure of the water hardness due to calcium and magnesium ions only? If hardness computations were specified to be derived from data obtained in site water calcium and magnesium determinations alone, confusion could be avoided and more accurate results obtained (APHA 1985). Site hardness values would thus not include contributions from other multivalent cations (e.g., iron, aluminum, manganese), would not rise above calcium + magnesium hardness values, or result in greater-than-intended site criteria when used in formulas. In this Biological opinion, what the Services refer to as hardness is the water hardness due to calcium + magnesium ions only.

The CTR should clearly state that to obtain a site hardness value, samples should be collected upstream of the effluent source(s). Clearly stating this requirement in the CTR would avoid the computation of greater-than-intended site criteria in cases where samples were collected downstream of effluents that raise ambient hardness, but not other important water qualities that affect metal toxicity (e.g., pH, alkalinity, dissolved organic carbon, calcium, sodium, chloride, etc.). Clearly, it is inappropriate to use downstream site water quality variables for input into criteria formulas because they may be greatly altered by the effluent under regulation. Alterations

in receiving water chemistry by a discharger (e.g., abrupt elevation of hardness, changes in pH, exhaustion of alkalinity, abrupt increases in organic matter etc.) should not result, through application of hardness in criteria formulas, in increased allowable discharges of toxic metals. If the use of downstream site water quality variables were allowed, discharges that alter the existing, naturally-occurring water composition would be encouraged rather than discouraged. Discharges should not change water chemistry even if the alterations do not result in toxicity, because the aquatic communities present in a water body may prefer the unaltered environment over the discharge-affected environment. Biological criteria may be necessary to detect adverse ecological effects downstream of discharges, whether or not toxicity is expressed.

The CTR proposes criteria formulas that use site water hardness as the only input variable. In contrast, over twenty years ago Howarth and Sprague (1978) cautioned against a broad use of water hardness as a "shorthand" for water qualities that affect copper toxicity. In that study, they observed a clear effect of pH in addition to hardness. Since that time, several studies of the toxicity of metals in test waters of various compositions have been performed and the results do not confer a singular role to hardness in ameliorating metals toxicity. In recognition of this fact, most current studies carefully vary test water characteristics like pH, calcium, alkalinity, dissolved organic carbon, chloride, sodium, suspended solids, and others while observing the responses of test organisms. It is likely that understanding metal toxicity in waters of various chemical makeups is not possible without the use of a geochemical model that is more elaborate than a regression formula. It may also be that simple toxicity tests (using mortality, growth, or reproductive endpoints) are not capable of discriminating the role of hardness or other water chemistry characteristics in modulating metals toxicity (Erickson *et al*. 1996). Gill surface interaction models have provided a useful framework for the study of acute metals toxicity in fish (Pagenkopf 1983; Playle *et al*. 1992; Playle *et al*. 1993a; Playle *et al*. 1993b; Janes and Playle 1995; Playle 1998), as have studies that observe physiological (e.g. ion fluxes) or biochemical (e.g. enzyme inhibition) responses (Lauren and McDonald 1986; Lauren and McDonald 1987a; Lauren and McDonald 1987b; Reid and McDonald 1988; Verbost *et al*. 1989; Bury *et al*. 1999a; Bury *et al*. 1999b). Even the earliest gill models accounted for the effects of pH on metal speciation and the effects of alkalinity on inorganic complexation, in addition to the competitive effects due to hardness ions (Pagenkopf 1983). Current gill models make use of sophisticated, computer-based, geochemical programs to more accurately account for modulating effects in waters of different chemical makeup (Playle 1998). These programs have aided in the interpretation of physiological or biochemical responses in fish and in investigations that combine their measurement with gill metal burdens and traditional toxicity endpoints.

The Services recognize and acknowledge that hardness of water and the hardness acclimation status of a fish will modify toxicity and toxic response. However the use of hardness alone as a universal surrogate for all water quality parameters that may modify toxicity, while perhaps convenient, will clearly leave gaps in protection when hardness does not correlate with other water quality parameters such as DOC, pH, Cl^- or alkalinity and will not provide the combination of comprehensive protection and site specificity that a multivariate water quality model could provide. In our review of the best available scientific literature the Services have found no

conclusive evidence that water hardness, by itself, in either laboratory or natural water, is a consistent, accurate predictor of the aquatic toxicity of all metals in all conditions.

Hardness as a predictor of copper toxicity: Lauren and McDonald (1986) varied pH, alkalinity, and hardness independently at a constant sodium ion concentration, while measuring net sodium loss and mortality in rainbow trout exposed to copper. Sodium loss was an endpoint investigated because mechanisms of short-term copper toxicity in fish are related to disruption of gill ionoregulatory function. Their results indicated that alkalinity was an important factor reducing copper toxicity, most notably in natural waters of low calcium hardness and alkalinity. Meador (1991) found that both pH and dissolved organic carbon were important in controlling copper toxicity to *Daphnia magna*. Welsh *et al*. (1993) demonstrated the importance of dissolved organic carbon in affecting the toxicity of copper to fathead minnows and suggested that water quality criteria be reviewed to consider the toxicity of copper in waters of low alkalinity, moderately acidic pH, and low dissolved organic carbon concentrations. Applications of gill models to copper binding consider complexation by dissolved organic carbon, speciation and competitive effects of pH, and competition by calcium ions, not merely water hardness (Playle *et al*. 1992; Playle *et al*. 1993a; Playle *et al*. 1993b). Erickson *et al*. (1996) varied several test water qualities independently and found that pH, hardness, sodium, dissolved organic matter, and suspended solids have important roles in determining copper toxicity. They also suggested that it may difficult to sort out the effects of hardness based on simple toxicity experiments. It is clear that these studies question the use of site calcium + magnesium hardness only as input to a formula to derive a criterion for copper because pH, alkalinity, and dissolved organic carbon concentrations are key water quality variables that also modulate toxicity. In waters of moderately acidic pH, low alkalinity, and low dissolved organic carbon, the use of hardness regressions may be most inaccurate. Also, it is not clear that the dissolved organic carbon in most or all waters render metals unavailable. This is because dissolved organic carbon from different sources may vary in both binding capacity and stability (Playle 1998).

Hardness as a predictor of silver toxicity: While there is strong evidence that ionic silver is the form responsible for causing acute toxicity in freshwater fish, recent science (Wood et al, 1999; Bruy eta al, 1999; Karen et al, 1999; Galvez and Wood, 1997; Hogstrand and Wood, 1998) challenges the EPA concept of hardness as having a large ameliorating effect on aquatic toxicity of silver. These studies indicate that chloride and dissolved organic carbon concentrations must be accounted for in the criterion formula for this metal. Bury *et al*. (1999) exposed rainbow trout to silver nitrate and measured physiological (Na^+ influx) and biochemical (gill Na^+/K^+-ATPase activity) endpoints, as well as silver accumulations in gills. They found that chloride and dissolved organic carbon concentrations, but not calcium hardness, ameliorated the inhibition of Na^+ influx and gill Na^+/K^+-ATPase activity. Dissolved organic carbon greatly reduced gill accumulations of silver through complexation. Chloride ion did not reduce gill accumulations of silver because it bound with free silver (Ag^+) and accumulated in gills as AgCl, but reduced toxicity because the AgCl did not enter chloride cells and disrupt ionoregulation.

Calcium, the hardness ion thought to modify metals toxicity to the greatest degree is, by itself, not

that protective in the case of silver. Karen *et al*. 1999 found DOC more important than hardness for predicting the toxicity of ionic silver in natural waters to rainbow trout, fathead minnows and *Daphnia magna*. These authors suggested incorporating an organic carbon coefficient into the silver criterion equation to enhance the site specificity of criterion. Wood et al (1999) noted chloride ion and DOC were influential in ameliorating silver toxicity and that in ammonia rich waters silver might be more than additively toxic with ammonia to fish.

Hardness as a predictor of cadmium toxicity: Our review of acute cadmium toxicity in fish indicates that calcium hardness does exhibit ameliorating effects (Reid and McDonald 1988; Verbost *et al*. 1989; Playle and Dixon 1993). However, most studies that manipulated hardness ions varied only calcium and so there is little evidence that magnesium ions ameliorate cadmium toxicity. Investigations of the differences between these two hardness constituents (Carroll *et al*. 1979; Davies *et al*. 1993) revealed that magnesium ions provide little or no protection against acute cadmium toxicity in fish. Hunn (1985) suggested that calcium binds to biological molecules in ways that magnesium does not, due to differences in the coordination geometry of the ions. Mechanistic studies of cadmium toxicity in fish reveal that cadmium inhibits enzyme-mediated calcium uptake in the gills (Verbost *et al*. 1989). Dissolved organic carbon, if present in sufficient concentrations and binding strengths, may also modulate cadmium toxicity. In natural waters hardness, pH, alkalinity, salinity, and temperature may also interact to affect cadmium toxicity but these factors may not always correlate to hardness measures at a given waterbed.

Loading

The Services are concerned that particulate metals discharges from municipal and industrial effluents will likely increase under the CTR proposed criteria. Current guidance for waste load allocation calculations (USEPA 1996b) consists of simple dilution formulations using effluent metal loads, receiving water flows, and dissolved-to-total metals ratios in the receiving waters. To illustrate our concerns, we expanded upon a hypothetical example contained in *The Metal Translator: Guidance For Calculating a Total Recoverable Permit Limit From a Dissolved Criterion* (USEPA 1996b). In this document, EPA provides a procedure for determining the concentration of total Cu that could be discharged in an effluent without exceeding the ambient criterion for dissolved Cu in the receiving water (i.e., a waste load allocation). In order to include additional metals in our analyses (not just Cu), we retained the assumptions of the EPA example for effluent flow, receiving stream flow, and ratio of dissolved metal to total metal in the receiving stream (f_d). For metals other than Cu, we assumed that the total metal in the receiving water, upstream of the discharge, was the same percentage of the National Toxics Rule (NTR) criterion as was assumed for Cu in the EPA example (~23 percent). For the 1992 NTR we assumed the same conditions as the EPA example but the total metal criteria was used.

Table 9 compares the concentration of total metals that could be discharged in an effluent without exceeding the ambient criterion for dissolved metals in the receiving water using: 1) total metal criteria from the 1992 NTR; 2) dissolved metal criteria from the CTR using a 40 percent dissolved-to-total metal ratio ($f_d = 0.4$) in the receiving water body; and 3) dissolved metal criteria

from the CTR using a 20 percent dissolved-to-total metal ratio (f_d = 0.2). The dissolved-to-total ratio of 0.4 is the same as that used in the EPA example and a ratio of 0.2 is not unusual for natural waters in California (CDFG 1997). It is evident that substantial increases in total metals would be permitted in this hypothetical discharge under proposed CTR criteria. If the dissolved fraction of total metals in the receiving water was 40 percent, then under the CTR, the total metal concentrations that would be allowed to be discharged would increase by 51 to 203 percent compared to the 1992 National Toxics Rule (Table 1). Nickel is the only metal under this scenario that would decrease (-21 percent). If the dissolved fraction of total metals in the receiving water was 20 percent, then under the CTR the total metal concentrations in allowable discharge would increase by 78 to 524 percent, including nickel (78 percent).

It also appears that as the fraction of particulate metal in the receiving water increases, the allowable discharge of particulate metals will increase, rather than decrease. The Services expect that increases similar to our examples would occur in allowable TMDLs under CTR criteria because a TMDL is the instream total metal concentration that equates to the dissolved metal criteria concentration (USEPA 1996b). Under the CTR, total metal discharges may increase as long as the dissolved criteria are not exceeded. Economic analyses of the draft CTR performed by the EPA and SWRCB (1997) show that implementing the new CTR criteria will decrease discharger costs by 50 percent because of "less stringent effluent limitations that result [from] the use of site-specific translators." Therefore, it would be incorrect to assume that TMDLs limit total metal loadings simply because they are expressed as total metal concentrations. Moreover, increases in permitted, point-source metal discharges will be incremental to discharges from agricultural or urban non-point sources, which are largely uncontrolled through the discharge-permitting process. Metals criteria based only on dissolved concentrations provide little in the way of incentives for reducing non-point sources, which are largely particulate forms. The Services are concerned that metals criteria based on dissolved concentrations in the absence of sediment criteria linked to total metals will not effectively prevent sediment contamination by metals and may lead to increased allowable loads of metals to sediments. The dissolved approach ignores the fact that water is more than a chemical medium; it also physically delivers metals to the sediments.

The Services believe that the CTR proposed formula-based metal criteria is not protective of threatened or endangered aquatic species because total metal discharges will likely increase and the criteria development methods do not adequately consider the environmental fate, transport, and transformation of metals in natural environments.

Table 9. Comparison of total metal concentrations permitted in a hypothetical point-source discharge under the 1992 National Toxics Rule that regulated metals on a total basis and the 1997 California Toxics Rule that proposes to regulate metals on a dissolved basis. The CTR concentrations are based on a receiving waterbed's percent dissolved to total metals of 40 and 20 percent. Values in parentheses are percent increase over 1992 NTR. Values are in µg/L total metal.

Receiving Water Percent Dissolved Metals	As	Cd	Cr (III)	Cr (VI)	Cu	Pb	Hg	Ni	Ag	Zn
NTR Total	973	11	1,487	43	48	230	7	3,835	9	318
CTR 40 percent	2,561 (163)	33 (203)	4,150 (179)	122 (182)	100 (106)	488 (112)	10 (51)	3,043 (-21)	26 (179)	888 (179)
CTR 20 percent	5,311 (446)	67 (524)	8,588 (478)	251 (483)	208 (331)	1,011 (339)	22 (219)	6,831 (78)	54 (478)	1,835 (476)

The Services find that the regulation of metals on a dissolved basis using the formulas proposed by the EPA in the CTR does not assure adequate protection of threatened or endangered species and their potential for exposure to dissolved and particulate metals in the water column because:

1. Criteria are based on toxicity tests that expose test organisms to metal concentrations with very low contributions from particulate metals and do not assess exposures under environmentally realistic conditions;

2. Particulate metals have been removed from the equation even though chemical, physical, and biological activity can cause these metals to become bioavailable. While the Services agree that dissolved metal forms are more toxic, this is not equivalent to saying that metals in the particulate fraction are not toxic, will not become toxic, are not being exposed to organisms, and do not require criteria guidance by the EPA;

3. Toxicity tests do not assess whether the toxic contributions of particulate metals are negligible when particulate concentrations are great and dissolved concentrations are at or near criteria levels;

4. The proposed criteria have the potential to significantly increase the discharge of total metal loads into the environment even though dissolved metal criteria are being met by a discharger;

5. The role of major cations (sodium, potassium), anions (nitrate, sulfate, chloride), and other water quality parameters (pH, temperature, dissolve organic matter) that modify metal toxicity may not be assumed to be negligible, thus hardness alone does not fully address site water effects

on toxicity;

6. The regulation of metals on a dissolved basis ignores the fact that water is more than a chemical medium, it also physically delivers metals to the sediments;

7. Larger databases with a wider range of test species used to derive the criteria can be nullified by use of smaller databases with fewer test species to adjust criteria on a site-specific basis via WER and CF translator determinations that use ratios which can greatly modify the final criteria; and

8. Aquatic criteria based on the dissolved metal fraction without concurrent wildlife criteria and sediment criteria fail to address a wide variety of exposure scenarios and effects such as bioaccumulation through the diet and synergism.

For these reasons the Services believe that the proposed formula-based method for developing metal criteria is not sufficiently protective of threatened or endangered aquatic species.

Metal Hazards to Aquatic Organisms

Sources

Eisler's series of synoptic reviews, EPA's criteria documents, Sorensen (1991), and Moore and Ramamoorthy (1985) provide a good summary of sources, pathways, and toxic effects of these metals. Metals in general are widely distributed and frequently, (as in the case of cadmium, copper, lead, and zinc) are found in the same ore deposits. Thus, activities such as mining can be a source of several metals at once. Metals are rarely found alone in discharges or the environment. Several metals are frequently associated with mining discharges, industrial discharges, and stormwater runoff. A variety of inorganic and organic forms of each metal are found in the environment and toxicity among these compounds varies widely.

There is a multitude of uses for these metals in the economy. Past and current uses include the production of numerous alloys, pigments, printing, wood preservatives, batteries, pesticides, electronics, electroplating, plastic stabilizers, tanning, furnaces, dyes, wiring, roofing, anticorrosion, plumbing, solders, ammunitions, gasoline additives, and currency.

Pathways

Because of the wide variety of uses, these metals can and will enter the environment through many pathways. The most direct routes are through acid mine drainage from active and abandoned mines and point-source discharges from industrial activities such as plating, textile, tanning, and steel industries. Municipal waste water treatment plants and urban runoff are also significant source of metals to the environment. Arsenic, copper, and zinc used as pesticides and wood preservatives enter the environment via drift, erosion, surface runoff, and leaching. Copper used

as an aquatic herbicide is directly applied to the water under controlled situations. Particulate metals from combustion and dust can be transported through the air.

Metals can enter the aquatic environment in a dissolved form or attached to organic and inorganic particulate matter. The amount of metal in the dissolved versus particulate form in natural waters can vary greatly, but the particulate form is usually found in greater concentrations. Metals can flux between different states and forms in an aquatic environment due to changes in pH, temperature, oxygen, presence of other compounds, and biological activity. These transformations can occur within and between water, sediment, and biota as the cycles of nature change.

As dissolved metals in the water, the most direct pathway to aquatic organisms is via the gills. Dissolved metals are also directly taken up by bacteria, algae, plants, and planktonic and benthic invertebrates. The dissolved forms of metals can adsorb to particulate matter in the water column and enter organisms through various routes. Metals adsorbed to particulates can also be transferred across the gill membranes (Lin and Randall 1990; Playle and Wood 1989; Sorensen 1991; Wright *et al*. 1986). Planktonic and benthic invertebrates can ingest particulate metals from the water column and sediments and then be eaten by other organisms. Thus, dietary exposure is a significant source of metals to aquatic and aquatic dependent organisms.

Although metals bound to sediments are generally less bioavailable to organisms, they are still present, and changes in the environment (e.g., dredging, storm events, temperature, lower water levels, biotic activity) can alter the bioavailability of these metals. The feeding habits of fish can determine the amount of uptake of certain metals. Piscivorous fish are exposed to different levels of metals than omnivorous and herbivorous fish. For example, copper is more commonly found in herbivorous fish than carnivorous fish from the same location (Mathis and Cummings 1971). In general, these metals do not biomagnify in the food chain as do mercury or selenium, thus impacts to resources tend to be limited to aquatic organisms.

General Toxicity of Metals

The toxicity of metals varies greatly depending on the chemical form and valence. Trivalent arsenic and hexavalent chromium are more toxic than other forms of arsenic and chromium, while chelated forms of metals are less toxic than the unbound ions. The various metals can have a wide variety of effects on organisms. They can cause enzyme inhibition due to reactions with the sulfhydryl groups of proteins. Some metals such as cadmium will compete with essential metals such as zinc for enzyme binding sites. Metal exposure can result in damage to gill and gut tissues, disrupt nervous system operation, and alter liver and kidney functions. Some metals can affect olfactory responses which are important to migrating salmonid species. Elevated metal concentrations can cause growth inhibition and impaired reproduction resulting in decreased primary production. An alteration of primary production can then impact growth and survival farther up the foodchain. Impacts from metal contamination can shift species composition and abundance towards more pollution-tolerant species. Copper is highly toxic to most freshwater invertebrates with LC 50s as low as 6 µg/L (Moore and Ramamoorthy 1984). The California

freshwater shrimp recovery plan notes this species is at particular risk from copper exposures relative to non-point sources associated with dairy operations and cow foot-baths using copper based compounds (USDI-FWS 1997a).

The toxicity of each metal to different organisms varies greatly. Copper is generally more toxic to aquatic organisms than the other metals. Complex synergistic effects among the metals can occur as well as antagonistic effects. The toxicity of metals can be altered by hardness, salinity, alkalinity, pH, and temperature. For most of the metals in the proposed rule, the criteria are formula based and hardness dependent because increasing hardness decreases the toxicity of the metal.

Particulate Toxicity

In the biological evaluation for the CTR, EPA determined that exposures to ambient concentrations of dissolved metals at or below the proposed CTR aquatic life criteria are unlikely to adversely affect threatened or endangered aquatic organisms (USEPA 1997a). While the CTR criteria proposed for metals are based on the dissolved fractions of these metals only, aquatic organisms in natural waters are exposed to additional, waterborne, particulate metal forms. As discussed in the CF section, the CTR will likely increase particulate metal loading even though dissolved criteria are being met. Dredging and disposal operations can result in substantial suspension and re-suspension of particulates in the water column, including those contaminated with metals.

Through respiratory uptake, aquatic organisms are exposed to metals in addition to those measured in the dissolved fraction of ambient waters. As fish ventilate, a nearly continuous flow of water passes across their gills (Moyle and Cech 1988) and particulate metals suspended in the water column may become entrapped. At the lowered pHs occurring near gill surfaces (Lin and Randall 1990; Playle and Wood 1989; Wright *et al.* 1986) entrapped particulate metals may release soluble metal ions (Sorensen 1991), which are the forms EPA considers most bioavailable and efficiently taken up by aquatic organisms (USEPA 1993a,1997a). Although most research has been done on particulate exposures to fish gills (primarily salmonids), it is reasonable to conclude that other fish and gill breathing organisms are affected in the same way.

Newly developed models seem well suited to assessments of the toxic contribution from suspended particulate metals and could be used to establish safe levels that do not substantially increase respiratory exposures. A panel of toxicologists has recently reviewed metals bioavailability and criteria issues and recommended replacing the current EPA approach to acute criteria development with a mechanistic approach such as a fish gill model (Bergman and Dorward-King 1997). Gill-model approaches have been used to successfully investigate how metal binding at fish gills is influenced by water hardness, pH, alkalinity, and dissolved organic carbon (Playle and Dixon 1993), as well as to estimate how effectively the gill competes with abiotic ligands for metals (Playle *et al.* 1993).

The Services believe that the proposed EPA metals criteria in the CTR for aquatic life should not exclude particulate forms of any metal, unless and until EPA demonstrates that exposures of threatened or endangered species to these contaminants are unlikely to cause adverse effects in natural waters.

Dietary Exposure

A biologically significant pathway for exposures of aquatic organisms to metals is through consumption of contaminated aquatic detritus, plants, invertebrates, and other food items. EPA has not assessed whether the food base of aquatic organisms may accumulate excessive metal residues under CTR proposed criteria. As the CTR preamble quotes from the CWA and EPA's 1985 guidelines, a criterion is the "highest concentration of a substance in water which does not present a significant risk to the aquatic organisms in the water and their uses." Their uses include "consumption by humans and wildlife." Certainly, an ecologically significant use of aquatic invertebrates is their consumptive use by fish. Invertebrates may accumulate appreciable body burdens of metals in aquatic systems and are prey consumed by salmonids and other fish species (Anderson 1977; Cain *et al*. 1992; Cain *et al*. 1995; Clements *et al*. 1994; Dallinger 1994; Elwood *et al*. 1976; Gerhardt and Westermann 1995; Ingersoll *et al*. 1994; Kiffney and Clements 1993; Luoma and Carter 1991; Lynch *et al*. 1988; McKnight and Feder 1984; Moore *et al*. 1991; Phillips 1978; Rainbow and Dallinger 1993; Smock 1983; Smock 1983a; Timmermans 1993; Saiki 1995; Zanella 1982; Moyle 1976; Saiki 1995).

The regulation of water quality criteria on a dissolved basis, as EPA proposes, does not consider particulates, sediment, and dietary exposure routes. In a recent experiment (Woodward *et al*. 1994) age-0 rainbow trout that were held in clean water and fed a diet of metals-contaminated invertebrates (for 91 days) exhibited reduced survival and growth. After 91 days, whole-body metal concentrations were similar to those in trout inhabiting the stream where the contaminated invertebrates were collected. In concurrent treatments, trout exposed to waterborne metals (at concentrations meeting criteria established by the EPA) and fed a diet of uncontaminated invertebrates exhibited no reductions in survival or growth. These results and those of similar studies of diet-borne metal exposures to salmonids collectively suggest that to reduce dietary hazards to salmonids, water quality criteria should protect invertebrate forage from excessive metal residue accumulations (Dallinger and Kautzky 1985; Dallinger *et al*. 1987; Farag *et al*. 1994; Giles 1988; Harrison and Klaverkamp 1989; Harrison and Curtis 1992; Miller *et al*. 1993; Mount *et al*. 1994; Thomann and Harrison 1997; Spry *et al*. 1988; Woodward *et al*. 1995).

The Services believe that without due consideration of dietary exposure of metals to aquatic organisms, the proposed CTR criteria for metals are not protective of threatened and endangered aquatic species. Criteria that are not protective of aquatic invertebrates from contamination and result in subsequent loss of beneficial use by fish and other aquatic organisms are not consistent with the CWA, nor are they protective of listed invertebrates considered in this biological opinion.

Bioaccumulation

As discussed throughout the formula based metals section, organisms are exposed to metals through many routes. These metals do bioaccumulate in the lower trophic levels of aquatic systems (Moore and Ramamoorthy 1984). The Services understand that EPA criteria development guidelines include a component designed to assure that the water quality criterion for a substance is sufficiently low that residue accumulations will not impair the use of aquatic organisms (USEPA 1985c). Data from residue studies are to be considered alongside acute and chronic toxicity data in the criteria development process (USEPA 1985c). However, it appears that the proposed metals criteria are based solely on results of aquatic toxicity tests (USEPA 1997c), where metal exposures occur only across gills or other respiratory surfaces. This is because toxicity tests used to develop the criteria are performed with controlled laboratory water with little particulate metals and do not include realistic dietary or other exposures.

Criteria documents for metals include the discussion of bioaccumulation studies but final criteria are based on acute and chronic toxicity studies. EPA has not considered results of investigations, similar to the studies discussed in the dietary exposure section, which indicate that exposures of salmonids to metals-contaminated invertebrate diets may result in adverse effects. Because EPA is now proposing criteria on a dissolved basis, and for the many reasons discussed throughout the formula-based metal discussion, bioaccumulation becomes even more important in evaluating the protectiveness of those criteria. A panel of toxicologists has recently reviewed metals bioavailability and criteria issues and recommended that ambient water criteria development include a tissue residue/toxicity model (Bergman and Dorward-King 1997).

The Services believe that without due consideration of the bioaccumulation potential of metals in aquatic systems the proposed CTR criteria for metals are not protective of threatened and endangered aquatic species.

Summary of Metal Criteria Effects to Listed Species

In summary, the effects of metals may be generalized to include: central nervous system disruption, altered liver and kidney function, impaired reproduction, decreased olfactory response, delayed smoltification, impaired ability to avoid predation and capture prey, growth inhibition, growth stimulation, changes in prey species community composition increasing foraging budgets, and lethality. The Services believe that all ESUs and runs of coho and chinook salmon and steelhead trout, Lahontan cutthroat trout, Paiute cutthroat trout, Little Kern golden trout, delta smelt, Sacramento splittail, Mohave tui chub, Lost River sucker, Modoc sucker, shortnose sucker, tidewater goby, and unarmored threespine stickleback are likely to be adversely affected by concentrations of particulate and/or dissolved metals at or below those that would be allowable under criteria procedures provided in the proposed CTR.

EPA Modifications to Address the Services' April 9, 1999 draft Reasonable and Prudent Alternatives for Dissolved Metals:

The above effect analysis evaluates the draft CTR as originally proposed in August of 1997. EPA has agreed by letter dated December 16, 1999, to modify its action for metals criteria per the following to avoid jeopardizing listed species.

A. *"By December of 2000, EPA, in cooperation with the Services, will develop sediment criteria guidelines for cadmium, copper, lead, nickel and zinc, and by December of 2002, for chromium and silver. When the above guidance for cadmium, copper, lead, nickel and zinc is completed, Region 9, in cooperation with the Services, will draft implementation guidelines for the State of California to protect federally listed threatened and endangered species and critical habitat in California."*

B. *"EPA, in cooperation with the Services, will issue a clarification to the Interim Guidance on the Determination and Use of Water-Effect Ratios for Metals (USEPA 1994) concerning the use of calcium-to-magnesium ratios in laboratory water, which can result in inaccurate and under-protective criteria values for federally listed species considered in the Services' opinion. EPA, in cooperation with the Services, will also issue a clarification to the Interim Guidance addressing the proper acclimation of test organisms prior to testing in applying water-effect ratios (WERs). "*

C. *"By June of 2003, EPA, in cooperation with the Services, will develop a revised criteria calculation model based on best available science for deriving aquatic life criteria on the basis of hardness (calcium and magnesium), pH, alkalinity, and dissolved organic carbon (DOC) for metals. This will be done in conjunction with "Other Actions A." below."*

D. *"In certain instances, the State of California may develop site-specific translators, using EPA or equivalent state/tribe guidance, to translate dissolved metals criteria into total recoverable permit limits. A translator is the ratio of dissolved metal to total recoverable metal in the receiving water downstream, from a discharge. A site-specific translator is determined on site-specific effluent and ambient data."*

"Whenever a threatened or endangered species or critical habitat is present within the geographic range downstream from a discharge where a State developed translator will be used and the conditions listed below exist, EPA will work, in cooperation with the Services and the State of California, to use available ecological safeguards to ensure protection of federally listed species and/or critical habitat. Ecological safeguards include: (1) sediment guidelines; (2) biocriteria; (3) bioassessment; (4) effluent and ambient toxicity testing; or (5) residue-based criteria in shellfish."

"Conditions for use of ecosystem safeguards:

1. A water body is listed as impaired on the CWA section 303(d) list due to elevated

metal concentrations in sediment, fish, shellfish or wildlife; or,

2. A water body receives mine drainage; or,

3. Where particulate metals compose a 50% or greater component of the total metal measured in a downstream water body in which a permitted discharge (subject to translator method selection) is proposed and the dissolved fraction is equal to or within 75% of the water quality criteria."

"Whenever a threatened or endangered species is present downstream from a discharge where a State developed translator will be used, EPA will work with the permitting authority to ensure that appropriate information, which may be needed to calculate the translator in accordance with the applicable guidance, will be obtained and used. Appropriate information includes:

4. Ambient and effluent acute and chronic toxicity data;
5. Bioassessment data; and/or
6. An analysis of the potential effects of the metals using sediment guidelines, biocriteria and residue-based criteria for shellfish to the extent such guidelines and criteria exist and are applicable to the receiving water body."

"EPA, in cooperation with the Services, will review these discharges and associated monitoring data and permit limits, to determine the potential for the discharge to impact federally listed species and/or critical habitats. If discharges are identified that have the potential to adversely affect federally listed species and/or critical habitat, EPA will work with the Services and the State of California in accordance with procedures agreed to by the Agencies in the draft MOA published in the Federal Register at 64 FR 2755 (January 15, 1999) or any modifications to those procedures agreed to in a finalized MOA."

Other [EPA] Actions

A. "EPA will initiate a process to develop a national methodology to derive site-specific criteria to protect federally listed threatened and endangered species, including wildlife, in accordance with the draft MOA between EPA and the Services concerning section 7 consultations."

Services' Assumptions Regarding EPA's CTR Modifications for regulating dissolved metals that result in Removing Jeopardy to listed species.

FORMULA BASED METALS CRITERIA

The Services assume EPA sediment guidelines for cadmium, copper, lead, nickel and zinc will be in place by December 2000 and sediment guidelines for chromium and silver will be in place by December 2002. The Services assume that these guidelines when implemented will increase protection for federally listed species and critical habitat. We also assume sediment guidelines will be used to limit particulate metal loadings into aquatic ecosystems in California.

The Services assume that the revised guidance on the use of water effect ratios for metals will reduce chances for inaccurate or under protective criteria.

The Services assume that a revised criteria calculation model for metals based on more than hardness, (pH, alkalinity, DOC) will actually result in more accurately protective criteria for federally listed species. The Services assume that use of such a model will require the use of more water quality parameter data (in addition to hardness) from water bodies where criteria are applied and that this supporting information will decrease the likelihood of under protective criteria.

The Services assume the use of site specific translators in metals discharge permits will not be used to allow significant increases in metal loadings in water bodies with mine drainage, or where water bodies are listed as impaired due to metals where listed species may be effected by such increases.

The Services also assume that where particulate metals are being transported to sediments under EPA approved discharge permits, these sediment locations will not exceed EPA guidelines for metals in sediment, especially where these water bodies contain federally listed species or critical habitat.

The Services assume the use of "ecosystem safeguards" such as ambient and effluent toxicity testing, biocriteria, sediment guidelines, and tissue based criteria, will increase the protection afforded federally listed species where metals are regulated on a dissolved basis.

CUMULATIVE EFFECTS

Cumulative effects include the effects of future State, Tribal, local, or private actions that are reasonably certain to occur in the action area considered in this biological opinion. Future Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act.

Cumulative effects on aquatic species including bonytail chub, coho salmon (all California ESUs), delta smelt, desert pupfish, Lahontan cutthroat trout, little Kern golden trout, Lost River sucker, Modoc sucker, Mohave tui chub, Owens pupfish, Owens tui chub, Paiute cutthroat trout, razorback sucker, Sacramento splittail, shortnose sucker, steelhead trout (all California ESUs), tidewater goby, unarmored threespine stickleback, and chinook salmon (all California ESUs) and their designated critical habitat within the aquatic ecosystems considered in this biological opinion include:

1. Water management such as diversions, levee maintenance, channel dredging, channel enlargement, flood control projects, drainage pumps, diversion pumps, siphons, non-Federal pumping plants associated with water management in the Sacramento-San Joaquin Delta, intrusion of brackish water, continuing or future non-Federal diversions of water, flood flow releases, and changes in water management;

2. Introduction of non-native fish, wildlife and plants, hybridization with non-native fishes, inbreeding of small populations, and genetic isolation;

3. Discharges into surface waters including point source discharges (permitted), non-point source runoff (e.g., mining runoff), runoff from high-density confined livestock production facilities, runoff from copper sulfate foot baths associated with dairy farms, agricultural irrigation drainwater discharges (surface and subsurface), runoff from overgrazed rangelands, municipal and industrial stormwater discharges (permitted and non-permitted), release of contaminated ballast and spills of oil and other pollutants into enclosed bays, and illegal, non-permitted discharges;

4. Overfishing and overutilization for scientific, commercial, and educational purposes;

5. Wildland fires and land management practices such as timber harvest practices and improper rangeland management resulting in sedimentation of surface waters; and application of pesticides, herbicides, fungicides,, fumigants, fertilizers and other soil/water amendments, urban development, and conversion and reclamation of wetland habitats;

6. Recreational disturbances including water sports, illegal fishing, and off-road vehicle use.

Cumulative effects for the semi-aquatic, piscivorous, and terrestrial wildlife including, Aleutian Canada goose, bald eagle, California brown pelican, California clapper rail, California least tern, light-footed clapper rail, marbled murrelet, western snowy plover, Yuma clapper rail, southern sea otter, Arroyo toad, California red-legged frog, giant garter snake, San Francisco garter snake, Santa Cruz long-toed salamander, California freshwater shrimp, conservancy fairy shrimp, longhorn fairy shrimp, Riverside fairy shrimp, San Diego fairy shrimp, Shasta crayfish, vernal pool fairy shrimp, and vernal pool tadpole shrimp and their designated critical habitat considered in this biological opinion include:

1. Water management such as diversions, levee maintenance, channel dredging, channel enlargement, flood control projects, installation of pumps, wells, and drains, non-Federal pumping plants associated with water management in the Sacramento-San Joaquin Delta, intrusion of brackish water, continuing or future non-Federal diversions of water, flood flow releases, and changes in water management;

2. Introduction of non-native fish, wildlife and plants, inbreeding of small populations, and genetic isolation;

3. Discharges into surface waters including point source discharges (permitted), non- point source runoff (e.g., mining runoff), runoff from high-density confined livestock production facilities, agricultural irrigation drainwater discharges (surface and subsurface), runoff from overgrazed rangelands, municipal stormwater runoff, and illegal, release of contaminated ballast and spills of oil and other pollutants into enclosed bays, non-permitted discharges;

4. Overutilization for scientific, commercial, and educational purposes;

5. Logging, wildland fire and land management practices including fluctuations in agricultural land crop production, plowing, discing, grubbing, improper rangeland management, timber harvest practices, irrigation canal clearance and maintenance activities, levee maintenance, permitted and non-permitted use and application of pesticides, herbicides, fungicides, rodenticides, fumigants, fertilizers and other soil/water amendments, urban development, urban refuse disposal, land conversions, illegal fill of wetlands and conversion and reclamation of wetland habitats; and

6. Recreational disturbances, vandalism, road kills, off-road vehicle use, chronic disturbance, noise, disturbances from domestic dogs and equestrian uses.

The adoption of the CTR is certain to affect listed species dependent on the aquatic ecosystem. These effects are prolonged and pose significant threats to species already threatened or endangered throughout their range. Continued growth and development in the State of California is likely to exacerbate existing environmental conditions for species already in peril. It is the summation of the direct, indirect, and cumulative effects of the proposed action that the Services conclude are likely to adversely affect these species and their habitats throughout the State.

CONCLUSION

Findings of Not Likely to Jeopardize

After reviewing the current status of the species, the environmental baseline for the action area, the effects of EPA's proposed action and its modifications to the proposed action for selenium, mercury, PCP, cadmium, and formula based dissolved criteria and the cumulative effects, it is the Services' biological opinion that the promulgation of the CTR, as modified by EPA's December 16, 1999 letter, is not likely to jeopardize the continued existence of, or adversely modify critical habitats for species listed in Table 3. The Services reached these conclusions for the following reasons: (1) adverse effects associated with the modified proposed action will be sufficiently minimized by NPDES permit evaluation and early coordination and consultation with the Services on all other CWA programs subject to section 7 consultation; (2) the time frames and procedural commitments proposed by EPA in their December 16, 1999, letter provide assurance that future criteria will be adequately protective of listed species and critical habitat; and (3) that EPA will promulgate such criteria in a manner that will provide protection to listed species and/or critical